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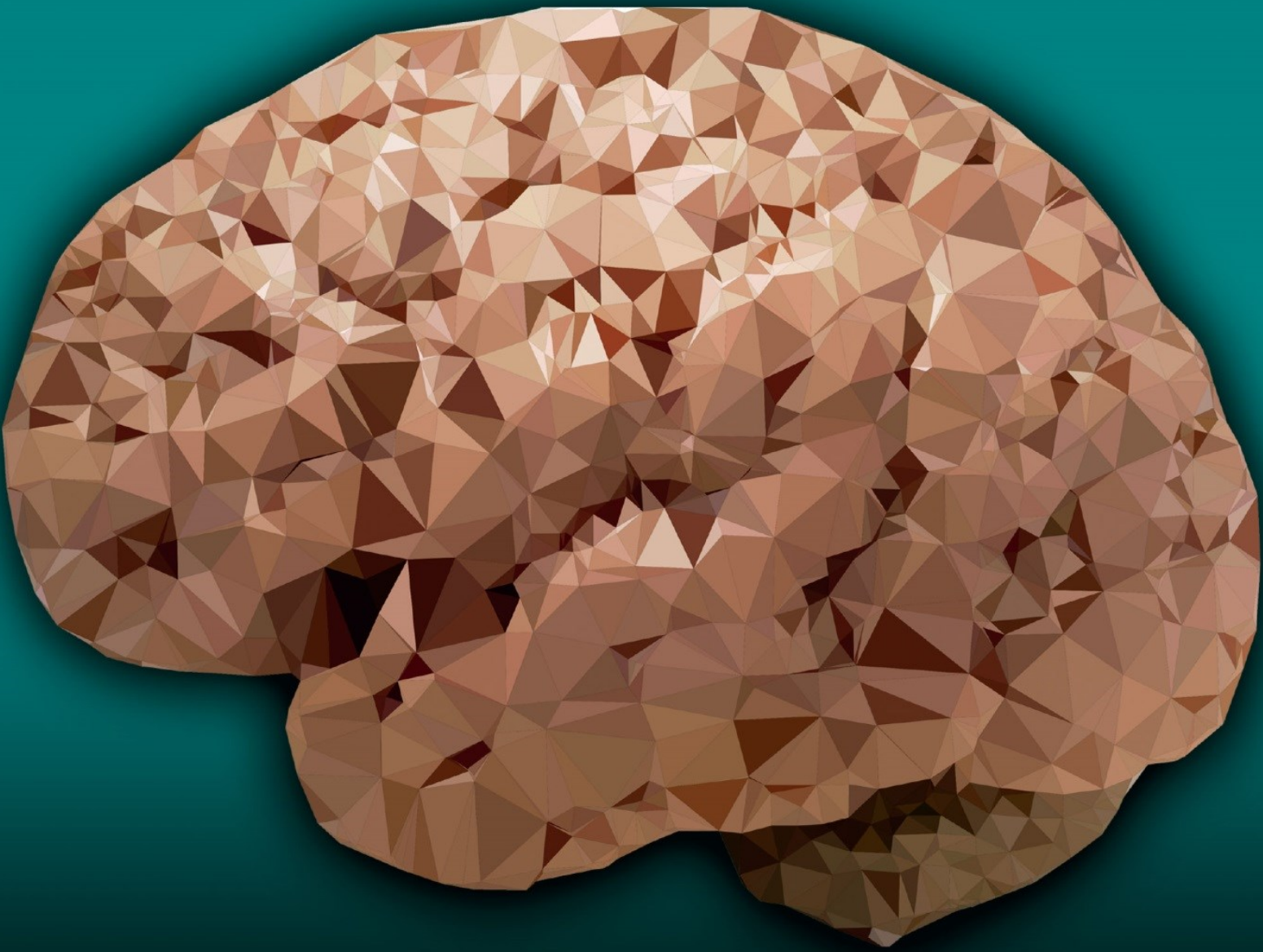
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Understanding adult ADHD

From genes to brain to behavior



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S E R I E S

Alberdingk Marten Henrick Onnink

Understanding adult ADHD

From genes to brain to behavior

A. Marten H. Onnink

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Understanding adult ADHD

From genes to brain to behavior

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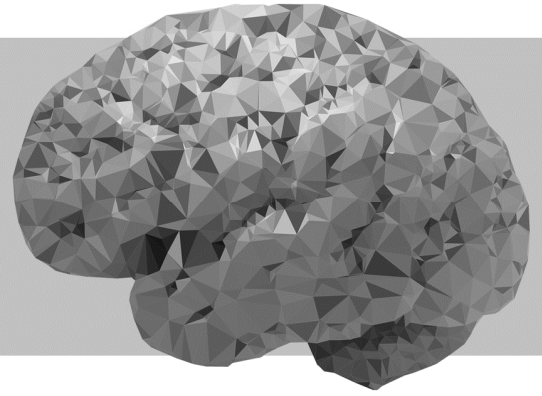
"Every place I go I take another place with me"

Wisconsin, Bon Iver

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General introduction

Attention deficit/hyperactivity disorder (ADHD) is a neuropsychiatric condition that is characterized by age-inappropriate levels of inattention, and/or hyperactivity/impulsivity that are associated with impairment of functioning. Originally, ADHD was considered only to affect children and was therefore not diagnosed in adults. Nearly two decades of research showed that in many children with ADHD, symptoms persist into adulthood. The persistence of ADHD into adulthood has been increasingly recognized and has developed into a research field of interest in its own right. However, considerably less is known about ADHD in adults relative to the childhood literature. This thesis is aimed at providing more insight into the pathophysiological profile of ADHD in adulthood.

In the following sections, I will give a more elaborate description of the clinical features and briefly review the genetic, neuroanatomical and cognitive literature of ADHD. Finally, I will provide the specific aims as well as a description of the study cohorts. In the subsequent chapters (*Chapters 2-6*), five empirical studies are described, addressing the specific aims of the thesis. These chapters are followed by a summary and a general discussion which provides the key findings and suggestions for future research (*Chapter 7*).

The clinical profile of ADHD

The course and persistence of ADHD

The prevalence rates of ADHD are estimated at 5-6% in childhood (Polanczyk et al., 2007). The core clinical symptoms of ADHD are divided into two domains, symptoms of inattention on the one hand and symptoms of hyperactivity and impulsivity on the other. For a clinical diagnosis of ADHD in children one needs to display six out of nine symptoms of inattention and/or six out of nine symptoms of hyperactivity/impulsivity, according to the *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition (DSM-5) (see Box 1) (American Psychiatric Association, 2013). Until the publication of the DSM-5 in 2013, there were no specific or official criteria for adult ADHD. The exact cutoff point for adult ADHD is now defined at five symptoms, which is in accordance with the existing literature (Kessler et al., 2010; Kooij et al., 2005). These symptoms must have emerged before the age of twelve years and have persisted for at least 6 months. They must also be manifested “to a degree that is inconsistent with developmental level and that negatively impacts directly on social and academic/occupational activities”.

With increasing age from childhood into adulthood, both inattentive and hyperactive/impulsive symptoms decline, although the latter much more abruptly (Biederman et al., 2000). The clinical presentation of ADHD is also somewhat different from what is seen in children. In adults, symptoms of inattention include difficulty sustaining attention when reading or doing paperwork, distractibility and forgetfulness, poor concentration; poor time management, difficulty finishing tasks, and misplacing things. Symptoms of hyperactivity can present as feelings of restlessness or of being overwhelmed, talking excessively; impulsivity is presented as changing jobs impulsively, driving too fast, being involved in traffic accidents, and irritability (Adler and Cohen, 2004). Also, the inability to plan and organize one’s activities is more prominent in adults than children with ADHD

(Haavik et al., 2010). A meta-analysis found that, of children diagnosed with ADHD during childhood, more than 50% will continue to be symptomatic and 15% still meet criteria for the full clinical disorder (Faraone et al., 2006). Prevalence rates of ADHD in adulthood are estimated to be between 2.5% and 4.9% (Simon et al., 2009). A recent study of lifetime prevalence of ADHD in The Netherlands reported childhood ADHD at a prevalence of 2.9%, and 70% of the affected children still meeting full ADHD criteria in adulthood (Tuithof et al., 2014). Importantly, recent studies suggest that ADHD is not always a stable diagnosis in adulthood (Karam et al., 2015), and that there even might be a form of adult ADHD that occurs without a history of childhood ADHD (Moffitt et al., 2015).

Altogether, ADHD is one of the most common mental health disorders (Kessler et al., 2005) and an increased number of adult patients in psychiatry is expected due to increased recognition and awareness (Asherson et al., 2007). Already in 2010, it was estimated that as many as 11 million adults have ADHD in the United States (Barkley et al., 2010). The problems associated with ADHD have an impact on work performance and absence, medical consumption, traffic and other accidents, and criminality (Matza et al., 2005). The annual costs of productivity loss at work were estimated on the basis of these findings at between 67 and 116 billion dollars in the United States (Biederman and Faraone, 2006).

Box 1 DSM-5 Diagnostic criteria for ADHD*

A persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development, as characterized by (1) and/or (2):

1. Inattention: Six (or more) of the following symptoms have persisted for at least 6 months to a degree that is inconsistent with developmental level and that negatively impacts directly on social and academic/occupational activities:

Note: The symptoms are not solely a manifestation of oppositional behavior, defiance, hostility, or failure to understand tasks or instructions. For older adolescents and adults (age 17 and older), at least five symptoms are required.

- a. Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or during other activities (e.g., overlooks or misses details, work is inaccurate).
- b. Often has difficulty sustaining attention in tasks or play activities (e.g., has difficulty remaining focused during lectures, conversations, or lengthy reading).
- c. Often does not seem to listen when spoken to directly (e.g., mind seems elsewhere, even in the absence of any obvious distraction).
- d. Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (e.g., starts tasks but quickly loses focus and is easily sidetracked).
- e. Often has difficulty organizing tasks and activities (e.g., difficulty managing sequential tasks; difficulty keeping materials and belongings in order; messy, disorganized work; has poor time management; fails to meet deadlines).
- f. Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (e.g., schoolwork or homework; for older adolescents and adults, preparing reports, completing forms, reviewing lengthy papers).
- g. Often loses things necessary for tasks or activities (e.g., school materials, pencils, books, tools, wallets, keys, paperwork, eyeglasses, mobile telephones).
- h. Is often easily distracted by extraneous stimuli (for older adolescents and adults, may include

unrelated thoughts).

i. Is often forgetful in daily activities (e.g., doing chores, running errands; for older adolescents and adults, returning calls, paying bills, keeping appointments).

2. Hyperactivity and impulsivity: Six (or more) of the following symptoms have persisted for at least 6 months to a degree that is inconsistent with developmental level and that negatively impacts directly on social and academic/occupational activities:

Note: The symptoms are not solely a manifestation of oppositional behavior, defiance, hostility, or a failure to understand tasks or instructions. For older adolescents and adults (age 17 and older), at least five symptoms are required.

a. Often fidgets with or taps hands or feet or squirms in seat.

b. Often leaves seat in situations when remaining seated is expected (e.g., leaves his or her place in the classroom, in the office or other workplace, or in other situations that require remaining in place).

c. Often runs about or climbs in situations where it is inappropriate. (Note: In adolescents or adults, may be limited to feeling restless.)

d. Often unable to play or engage in leisure activities quietly.

e. Is often “on the go,” acting as if “driven by a motor” (e.g., is unable to be or uncomfortable being still for extended time, as in restaurants, meetings; may be experienced by others as being restless or difficult to keep up with).

f. Often talks excessively.

g. Often blurts out an answer before a question has been completed (e.g., completes people’s sentences; cannot wait for turn in conversation).

h. Often has difficulty waiting his or her turn (e.g., while waiting in line).

i. Often interrupts or intrudes on others (e.g., butts into conversations, games, or activities; may start using other people’s things without asking or receiving permission; for adolescents and adults, may intrude into or take over what others are doing).

In addition, the following conditions must be met:

Several inattentive or hyperactive-impulsive symptoms were present prior to age 12 years.

Several inattentive or hyperactive-impulsive symptoms are present in two or more settings (e.g., at home, school, or work; with friends or relatives; in other activities).

There is clear evidence that the symptoms interfere with, or reduce the quality of, social, academic, or occupational functioning.

The symptoms do not occur exclusively during the course of schizophrenia or another psychotic disorder and are not better explained by another mental disorder (e.g., mood disorder, anxiety disorder, dissociative disorder, personality disorder, substance intoxication or withdrawal).

Specify whether:

314.01 (F90.2) Combined presentation: If both Criterion A1 (inattention) and Criterion A2 (hyperactivity-impulsivity) are met for the past 6 months.

314.00 (F90.0) Predominantly inattentive presentation: If Criterion A1 (inattention) is met but Criterion A2 (hyperactivity-impulsivity) is not met for the past 6 months.

314.01 (F90.1) Predominantly hyperactive/impulsive presentation: If Criterion A2 (hyperactivity-impulsivity) is met and Criterion A1 (inattention) is not met for the past 6 months.

Specify if:

In partial remission: When full criteria were previously met, fewer than the full criteria have been met for the past 6 months, and the symptoms still result in impairment in social, academic, or

occupational functioning.

Specify current severity:

Mild: Few, if any, symptoms in excess of those required to make the diagnosis are present, and symptoms result in no more than minor impairments in social or occupational functioning.

Moderate: Symptoms or functional impairment between “mild” and “severe” are present.

Severe: Many symptoms in excess of those required to make the diagnosis, or several symptoms that are particularly severe, are present, or the symptoms result in marked impairment in social or occupational functioning.

*From the DSM-IV-TR to DSM-5, some changes in the criteria were made: the age at which symptoms must first appear was changed from 7 to 12 years old, and the number of symptoms required for the diagnosis to apply for adults was specified as five (as opposed to six for children) (American Psychiatric Association, 2000, 2013).

A dimensional approach to ADHD

A clinical diagnosis of ADHD is a binary measure (present/absent), wherein three presentations of ADHD can occur (combined, predominantly inattentive and predominantly hyperactive-impulsive). However, ADHD is phenotypically a heterogeneous disorder since it is diagnosed when a patient exhibits a minimal number of observable items drawn from a larger list. Selecting six, seven, eight, or nine out of a list of nine elements in each of the two lists in Box 1 yields 130 possible combinations for each list, and in total there are 16,900 unique symptom sets that meet criteria for ADHD. For ADHD in adults, this number reaches even 65,536 when lowering the number of necessary symptoms to 5. More importantly, ADHD traits are continuously distributed in the general population and could be seen as the (behavioral) extreme end of this continuum in the general population (Larsson et al., 2012). Nowadays, this is increasingly recognized and leads to a shift from a categorical disease definition of ADHD to a more dimensional, quantitative approach (Coghill and Sonuga-Barke, 2012). This approach of reducing the complex heterogeneous phenotype of ADHD by using clinical dimensions already showed its potential. Recent studies show that this approach can facilitate the identification of ADHD genes (Bralten et al., 2013) and anatomical brain-behavior relationships (Hoogman et al., 2012).

Comorbidities of adult ADHD

Comparable to children with ADHD, comorbid disorders are frequently present in adults with ADHD (Kessler et al., 2006; McGough et al., 2005). It is estimated that 70–75% of adult patients with ADHD have at least one additional psychiatric diagnosis (Biederman, 2004; Kessler et al., 2006). Psychiatric comorbidity is considered to be an important factor for impairment and persistence of ADHD symptoms (Biederman et al., 2011; Biederman et al., 2012; Lara et al., 2009). Frequent comorbidities seen in ADHD are substance use disorders, anxiety and mood disorders, oppositional, defiant and conduct disorder and autism spectrum disorder (Anckarsater et al., 2006; Wilens et al., 2009). Recurring depressive episodes occur in up to 50% of adults with ADHD (McIntosh et al., 2009). The high prevalence of comorbidity often complicates the diagnostic process. For example, symptoms of ADHD and depression overlap in terms of concentration and sleep problems,

psychomotor agitation or slowness, and fatigue or loss of energy. However, symptoms in ADHD are chronic from childhood, while in a depressive episode, there is a period before and after the symptoms. In the combination of ADHD and depressed episodes, ADHD patients can distinguish between their normal self and the depressed episode (Kooij, 2012). Developmental disorders like autism spectrum disorders (ASD), Tourette and tic disorders, developmental delay and learning disorders have also frequently been reported as comorbid to ADHD (Buitelaar et al., 2011). In contrast to earlier editions of the DSM, the DSM-5 allows an inclusion of comorbid ADHD and ASD (American Psychiatric Association, 2013) which will likely boost research on the shared and specific pathways related to comorbid ASD and ADHD.

Gender differences in adult ADHD

ADHD in childhood is more prevalent in boys than in girls. In community-based samples of youth, a male to female ratio of about 3:1 was reported (Willcutt, 2012) and in clinical populations, this ratio climbs even higher to 9:1 (Gershon, 2002). The difference in prevalence rates for males and females diminishes in adulthood (Biederman et al., 1994; Biederman et al., 1993; Faraone et al., 2000b). Girls are probably underdiagnosed, which might be due to the fact that ADHD in girls is less well known among general practitioners and other health-care provider and therefore results in fewer referrals (Kooij, 2012). It has been reported that number of symptoms and lifetime comorbidity rates in ADHD do not vary by gender (Biederman et al., 2004), while other studies showed that women with ADHD were more likely to report higher rates of anxiety, mood, and eating disorders (Quinn, 2011).

Treatment of ADHD

There is as yet no cure for ADHD. The primary treatment for ADHD consists of using medication (either stimulants like methylphenidate/dextroamphetamine or nonstimulants like atomoxetine)(Faraone et al., 2004) often in addition to cognitive-behavioral therapies (Mongia and Hechtman, 2012). While psychostimulants constitute the most efficacious drugs in the treatment of ADHD it provide only symptomatic relief rather than it is curative and there is no evidence that these treatments have a positive effect on long term prognosis of ADHD (Buitelaar et al., 2011). Although some longer lasting follow-up studies reported beneficial effect of treatment on ADHD core symptoms (Bejerot et al., 2010; Torgersen et al., 2012; Wender et al., 2011).

In conclusion, ADHD is a descriptive term covering a clinically heterogeneous condition which is characterized by symptoms of inattention, hyperactivity and impulsivity that begins early in life and in some cases persists into adulthood. It is often associated with comorbidity such as substance abuse, depression, autism, and other problems and has a huge emotional and financial burden on society. Although research on adult females with ADHD continues to lag behind that on adult males with ADHD, there are indications that gender related

differences exist in comorbid conditions. As in childhood ADHD, medication remains a key component of treatment for adults with ADHD.

The neuroscience of ADHD

Nowadays, genetic, pharmacological, imaging, and animal model studies provide strong support for a neurobiological basis for ADHD. Still, there are currently no non-behavioral tests, either psychological or (neuro)biological that provide enough sensitivity and specificity to be used as a dichotomous diagnostic criterion and thus relies a diagnosis exclusively on subjective judgments (Faraone et al., 2014; Scassellati et al., 2012). Although, ADHD is not likely to be determined by any single construct, we still know relatively little about the exact causes and the mechanisms underlying ADHD. Moreover, compared with the childhood ADHD literature, there is fewer literature available on adults with ADHD. This thesis includes studies on a wide variety of issues on different levels including genetic, neurobiological and neuropsychological correlates of ADHD in adults. I will briefly introduce these topics in the following chapters and I will describe the endophenotype model.

The genetics of ADHD

The first studies reporting that hyperactivity runs in families appeared as early as the 1970's (Morrison and Stewart, 1971). Since then, twin and adoption studies showed that 76% of the variance for ADHD is explained by genetic factors (Faraone et al., 2005). The persistent form of ADHD in adults has been associated with comparable or even stronger genetic influences (Faraone et al., 2000a; Franke et al., 2012). Despite the high heritability of the disorder, it has been hard to find genes underlying the disorder. On one hand, this is due that ADHD is a complex trait which results from the phenotypical heterogeneity which is characterized by the described different symptom domains, comorbidities and by different neurobiological systems thought to be involved. On the other hand, ADHD is not caused by a single gene but rather by a number of genes at the same time, each contributing with small effect size. In contrast, rare genes with major effect size (e.g. CNVs) may play a role in a small proportion of cases as is discussed further below. Moreover, these genetic influences are likely to interact with environmental factors. Numerous approaches have been developed in the search of genes that influence ADHD. Early hypothesis-driven studies mainly focused on candidate genes known to affect aspects of the disease etiology such as dopaminergic and noradrenergic neurotransmission (*DAT1/SLC6A3*, *DRD1-4*, *NET1*, *ADRA2A*). Findings from these studies demonstrate that certain alleles (sequence variants) of some genes occur more frequently in patients with ADHD than in healthy controls (Gizer et al., 2009; Li et al., 2014). To make it even more complex, it is suggested that the genetic component of adult ADHD is partly different from the one observed in children (Franke et al., 2012). This is demonstrated by the dopamine transporter gene, *DAT1*, which has been studied most extensively in ADHD research. Meta-analyses show that the 10-repeat allele of this polymorphism is associated with ADHD in youth (Gizer et al., 2009), whereas the 9-repeat allele is associated with ADHD in adults (Franke et al., 2008; Franke et al., 2010). Longitudinal studies or studies including

both children and adult subjects (including persistent and remitted forms of ADHD) are therefore needed since the mechanism by which this gene increases disease risk remains largely unknown. Since not all aspect of the disease etiology in ADHD is known, the hypothesis-driven candidate gene based studies are likely to miss at least part of the genetic variance and cannot highlight novel biological mechanisms. Genome-wide association studies (GWAS) overcome this drawback by taking a data-driven approach and search for genetic association across the genome in large samples of cases and controls. GWAS studies typically focus on associations between single-nucleotide polymorphisms (SNPs) which are the most frequent type of variation in the human genome. Unfortunately, GWAS studies in ADHD have not identified any genome-wide significant results thus far (Franke et al., 2009). Recent findings, however, strengthens the hypothesis that individually rare copy number variations (CNVs) (too uncommon to detect in GWAS) encompassing relatively large regions of the genome might contribute to ADHD in children and adults with promising effect sizes (Franke et al., 2012; Li et al., 2014; Williams et al., 2012). Another informative approach has been to examine how top GWAS hits may fit into a genetic network that is biological plausible and serves a specific function. In ADHD, one such network may be involved in directed neurite outgrowth (Poelmans et al., 2011). Alternatively, since multiple genes with small effect sizes are assumed to play a role in ADHD, another approach was opposed to test the association of multiple variants in a pathway as a whole to increase the total explained phenotypic variance (Bralten et al., 2013). These new avenues in the identification of candidate genes may generate novel and interesting results for the genetic basis of ADHD.

Anatomical neuroimaging in ADHD

With the arrival of magnetic resonance imaging (MRI) in the late 1980s, the development of brain structure and function could now be investigated in vivo in a non-invasive manner. Structural MRI assesses the morphological aspects of the brain and at the moment there is a wealth of literature showing that ADHD is accompanied by volume reductions in total cerebral, corpus callosum (CC) and subcortical areas as shown by several meta-analyses (Ellison-Wright et al., 2008; Frodl and Skokauskas, 2012; Nakao et al., 2011; Valera et al., 2007). There are indications that differences in subcortical structures such as the putamen and caudate wane with increasing age and disappear in adulthood (Castellanos et al., 2002; Nakao et al., 2011). However, a longitudinal examination showed that typically developing controls exhibited continued expansion of surface area of the ventral striatum between 4 and 19 years, whereas children and adolescents with ADHD showed steady contraction. The ADHD group also showed surface area reductions of dorsal striatal regions which were detected in childhood and persisted into adolescence (Shaw et al., 2014a). These results are from the latest publication in a long line of landmark studies that are derived from the largest longitudinal study of brain structure in ADHD. A previous publication from this study showed that maturation of cortical areas, most markedly in the frontal lobes, were delayed in patients with ADHD (Shaw et al., 2007a). Moreover, they showed in another study that children with ADHD whose symptoms persisted into adulthood displayed higher rates of

cortical thinning across development when compared to healthy controls while individuals who remitted showed a slower rate of cortical thinning – and in some cases, even cortical thickening – than healthy controls. Put together, these results support a neurodevelopmental model for ADHD persistence proposing that the disorder is caused by subcortical neural dysfunction that is present early in life and remains relatively stable throughout the lifetime, potentially compensated by prefrontal cortex function in remitting forms of ADHD (Halperin and Schulz, 2006). Cross-sectional studies performed in adults indeed show smaller volumes in subcortical areas (Almeida Montes et al., 2010; Proal et al., 2011; Seidman et al., 2011), cortical areas (Biederman et al., 2008; Makris et al., 2007; Seidman et al., 2011; Seidman et al., 2006), anterior cingulate (Amico et al., 2011; Biederman et al., 2008; Makris et al., 2007; Seidman et al., 2006) and cerebellar areas (Biederman et al., 2008; Proal et al., 2011; Seidman et al., 2011). However, there are also some negative findings with respect to structural differences in frontal lobes and basal ganglia which are in conflict with the model of Halperin & Schulz (Ahrendts et al., 2011; Amico et al., 2011; Perlov et al., 2008).

A relatively recent neuroimaging technique is diffusion tensor imaging (DTI) which is used to study the microstructural organization of white matter tracts that connect gray matter regions. DTI is based on the property of water molecules to diffuse along fiber tracts which enables exploration of microstructural integrity of white matter (Konrad and Eickhoff, 2010; Le Bihan, 2003). Compared to the wealth of structural MRI in ADHD, relatively few DTI studies in ADHD exist although the number of studies has increased rapidly in the last years. This is illustrated by a quick search in PubMed using the keywords “ADHD” and “diffusion tensor imaging”. While this search yields now more than 85 results, ten years ago in 2004 the same keywords yielded only 4 studies. A recent meta-analysis in childhood, adolescent and adult ADHD suggests alterations in white matter integrity in a range of fiber pathways connecting cortical and subcortical regions thought to subserve cognitive functions implicated in ADHD (van Ewijk et al., 2012). The DTI literature is hampered by variations of age and medication history of participants across studies, as well as of the approaches used to analyze the DTI data (such as hypothesis-free whole brain approaches or hypothesis-driven manually selected regions of interest), making the integration of results somehow difficult. Moreover, the current literature lacks longitudinal DTI studies in ADHD, although it has been suggested that a history of childhood ADHD leaves a ‘mark’ on white matter that persists regardless of the course of ADHD in adulthood (Cortese et al., 2013; Shaw et al., 2015). However, this is dependent on the definition of a trait marker, since this no longer holds when a more stringent definition is used (Shaw et al., 2015).

For both structural MRI and DTI, the jury is still out as to where adult ADHD patients have altered brain tissue volume and/or altered white matter characteristics, and how these abnormalities are associated with the clinical profile. An important question that needs to be addressed when considering the continuity of neuroanatomical alterations into adulthood in ADHD is whether pharmacological treatment has an impact on structural changes across the life cycle. Interestingly, structural MRI several studies in adults with ADHD did not show a

reduction in total brain volume (Biederman et al., 2008; Hesslinger et al., 2002; Monuteaux et al., 2008; Perlov et al., 2008; Seidman et al., 2006). This is striking, given that a global reduction in total cerebral volume is a consistent finding in children/adolescents with ADHD (Valera et al., 2007). The failure to find such a volume reduction could be due to the effects of chronic treatment with stimulant medication. This possibility is based on two recent meta-analyses exploring gray matter volume in ADHD that both agree that stimulant use is associated with an absence of reductions of regional gray matter volumes, suggesting a normalizing effect of treatment on neural abnormalities (Frodl and Skokauskas, 2012; Nakao et al., 2011). Normalization of brainvolume and function of white matter, basal ganglia, anterior cingulate cortex, thalamus, and cerebellum with longterm stimulant treatment have also been described (Schworen et al., 2013; Sobel et al., 2010). However, a recent study could not confirm medication effects on brain volumes (Greven et al., 2015).

In the DTI literature, no study so far have specifically addressed the issue of medication effects. It has been suggested that white matter volume is susceptible to stimulant medication (Castellanos et al., 2002) although some DTI studies in children with ADHD did not report confounding effects of (stimulant) medication on their results (de Zeeuw et al., 2012; Hamilton et al., 2008; van Ewijk et al., 2014).

The neuropsychology of ADHD

The neuropsychological literature of ADHD is vast and numerous theories have been proposed over the years. Initially, researchers have sought for a single core cognitive deficit (e.g. behavioral inhibition) underlying ADHD (Barkley, 1997). However, comprehensive reviews contributed to a shift from this traditional core deficit view to multiple deficit theories (Nigg, 2005; Willcutt et al., 2005). These multiple deficit theories propose that there are distinct pathways to dysfunction, including executive function deficits, delay aversion, and timing problems (Castellanos et al., 2006; Castellanos and Tannock, 2002; Nigg and Casey, 2005; Sonuga-Barke, 2002; Sonuga-Barke et al., 2010). Recently, novel clustering algorithms have documented cognitive heterogeneity in both patients with ADHD and healthy control subjects. They show that heterogeneity in cognitive functions is present across groups and that most individuals could be classified into similar, distinct, neuropsychological profiles. (Coghill et al., 2014; Fair et al., 2012; van Hulst et al., 2014). Research into the neuropsychology of ADHD in adults have used similar or even the same neuropsychological tests employed with children with ADHD and often with comparable results (Boonstra et al., 2005; Hervey et al., 2004; Seidman, 2006). However, compared with childhood ADHD, studies on adults with ADHD are sparse and have assessed only a narrow range of neuropsychological functions.

One of the most common findings in ADHD is increased reaction time variability (RTV). RTV is the moment-to-moment fluctuation of performance in neuropsychological reaction time (RT) experiments. Initially, increased RTV was perceived as noise in the data but is now extensively studied in ADHD (Karalunas et al., 2014; Kofler et al., 2013; Kuntsi and Klein, 2012; Tamm et al., 2012). Increased RTV is currently considered by many to be a core

and stable feature of ADHD and is observed across other psychiatric disorders (Kofler et al., 2013; Tamm et al., 2012). Some theories that have been proposed hypothesize that increased RTV is explained by top-down mechanisms such as attentional control and arousal (Epstein et al., 2011). Alternatively, bottom-up theories hypothesize neurobiological mechanisms responsible for increased RTV such as reduced myelination of white matter tracts (Russell et al., 2006). Traditionally, RTV has been estimated using the standard deviation of reaction times (RTSD) which represents how data is spread around the mean value. This method does not recognize that RT distributions are often positively skewed and therefore misses a significant amount of specific information regarding the distribution of RT. During the last decade, new statistical approaches such as the ex-Gaussian analysis on RTs has provided enlightening results regarding the dynamic nature of RTV in ADHD. The meta-analysis by Kofler et al. (2013) recognized that increased RTSD in ADHD reflects RT in the exponential tail of the distribution, captured by the parameter '*tau*'. In their seminal paper on the ex-Gaussian variables in ADHD, Leth-Steensen et al. (2000) propose that abnormally slower reaction times (or *tau*) might represent infrequent occasional lapses in attention.

The endophenotype approach

Reviewing previous chapter's reveals that ADHD appears to have a complex etiology that is accompanied by great heterogeneity at multiple levels of analysis (e.g., genetic, neuroanatomical, neuropsychological and clinical level). Multiple factors including genetics and environmental factors with small individual effect appear to interact and cause neurobiological liability to ADHD. To gain more insight into the mechanisms leading from a genetic/biological basis of the disease to the full clinical phenotype, endophenotypes (or intermediate phenotypes) are considered a promising strategy (Faraone et al., 2014). Endophenotypes are (a) those characteristics of a disorder that are linked relatively closely to its neurobiological substrates than its clinical symptoms (Doyle et al., 2005) and (b) shares one or more of the same susceptibility genes with the condition it subtends (Gottesman and Gould, 2003). One of the assumptions is that the genetic root of endophenotypes is less complex than that of the disorder itself, due to their relative proximity to gene actions. As a result, they are hoped to confer greater statistical power to identify genetically driven neurobiological mechanisms than disease status alone. This theoretical model is illustrated in Figure 1. Neuroimaging measures such as structural MRI (Hulshoff Pol et al., 2006) and DTI (Jahanshad et al., 2013) are highly heritable suggesting strong genetic influences. Investigating the genetic influences on these brain measures is also known as imaging genetics, and have been offered as key endophenotypes for capturing underlying liability for ADHD (Dresler et al., 2014; Durston, 2010; Wu et al., 2014). In schizophrenia research, it has already been reported that risk variants indeed had larger effect sizes at the level of brain structure and brain function compared to effect sizes for behavioral traits (Rose and Donohoe, 2013). In the ADHD literature, a smaller volume of the basal ganglia has been found in both patients with ADHD and unaffected family members, which suggest that it is

associated with an increased risk of the disorder in families (Castellanos et al., 2002). In this respect, the *DAT1* gene is an interesting candidate which is also the most studied gene in relation to neuroimaging in ADHD. Two structural imaging studies showed that homozygosity for the ADHD risk allele (10-repeat) was associated with smaller caudate nucleus volume (Durstion et al., 2005; Shook et al., 2011). A longitudinal study did not reveal any effect of SLC6A3 genotype on cortical thickness (Shaw et al., 2007b). This demonstrates that the *DAT1* gene affects only regions where it is preferentially expressed (i.e. in the striatum) (Durstion et al., 2009). As is described earlier, a different variant of *DAT1* is associated with childhood ADHD (haplotype 10-6) and adult ADHD (haplotype 9-6). This proposes a differential association depending on age, and a role of *DAT1* in modulating the ADHD phenotype over life time. Imaging genetic studies on the effect of *DAT1* on striatal volumes including both children and adult subjects are therefore promising as they could elucidate a mechanism by which this gene increases disease risk.

As described above, using structural MRI as an endophenotype provides an excellent way to reduce the complexity and help us comprehend the biological meaning of these genetic variants in the etiology of ADHD. Moving away from the genetic level, a further understanding can be achieved by examining how structural changes in the brain are related to cognitive functions. Several neuroimaging studies started to use a dimensional approach (as described above) and investigate the relationship between neuroimaging measures and clinical measures. For example, it has been shown that brain volume correlates with severity of ADHD as measured with clinical symptoms (Castellanos et al., 2002; Hoogman et al., 2012) and this relationship has also been found for white matter abnormalities (Shaw et al., 2014b; van Ewijk et al., 2014). Furthermore, this approach can also be applied on cognitive measures. For example, studies in adults with ADHD showed that less gray matter in the right inferior frontal gyrus correlated with poorer outcomes in measures of processing speed, response inhibition and response variability (Depue et al., 2010; Pironti et al., 2014).

Neuropsychological measures are also known as potential ADHD endophenotypes (Rommelse et al., 2008). As it is unlikely that a single core neuropsychological deficit is underlying ADHD, parsing the phenotype along behavioral measures will tell us more about the neurobiological underpinnings. For this purpose, the ex-Gaussian parameter *tau* might be a good candidate. As shown above, increased *tau* is consistently found in ADHD with a clear signal related to attention problems (Kofler et al., 2013; Leth-Steensen et al., 2000). While reduced myelination has been hypothesized as a biological mechanism responsible for increased *tau* (Russell et al., 2006), a recent study indeed found that increased *tau* in childhood patients with ADHD was associated with reduced white matter integrity in the cingulum bundle (Lin et al., 2014). Despite the growing interest of ex-Gaussian RT analysis, studies investigating ex-Gaussian parameters and their neural correlates in adult patients are still lacking.

Identifying and characterizing anatomical and behavioral endophenotypes for ADHD will bring us closer to a better understanding of ADHD on different levels. Firstly, compared to clinical symptoms, endophenotypes are better suited to be used as quantitative trait loci

in future ADHD linkage and association studies, given their relative proximity to the underlying biology. Secondly, an important advantage of studying endophenotypes in ADHD is that they have the potential to overcome the heterogeneity observed in the behavioral phenotype. Neuropsychological endophenotypes can therefore lead to the identification and examination of more homogeneous subgroups of patients as suggested by the multiple deficit theories. Lastly, endophenotype promote our understanding of the precise neurobiological mechanisms underlying the disorder and its treatment, and opening the possibility of developing optimized behavioral and pharmacological treatment solutions for affected patients.

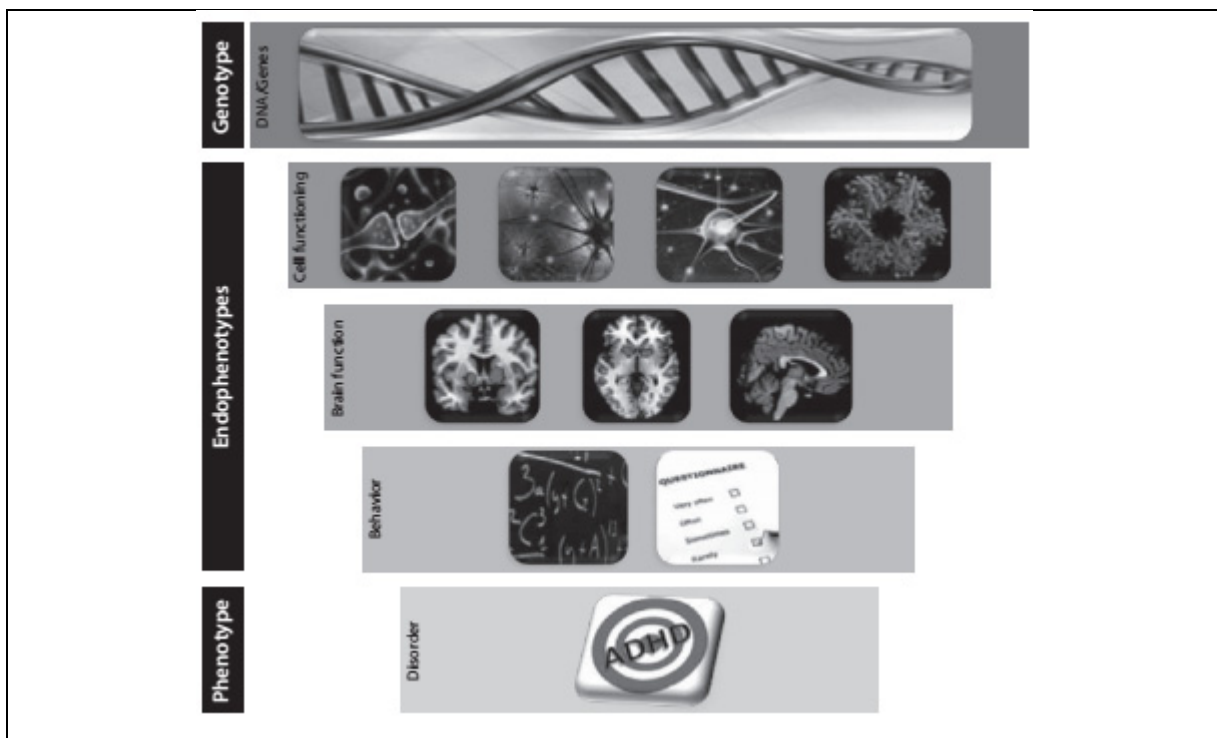


Figure 1. A systematic overview of the pathway between genes and a (psychiatric) disorder is displayed above. Endophenotypes are placed between the genotype (genetic variation related to the disorder) and the phenotype (observable characteristics of the disorder). Examples of endophenotypes are the size of, or activation in various brain structures.

*Adapted from (Hoogman, 2013).

The aim of this thesis

The overall aim of this thesis is to advance our knowledge of genetic, neuroanatomical and neurocognitive factors involved in adult patients with ADHD.

The first specific aim is to fill a gap in the literature by thoroughly exploring the association of the clinical ADHD phenotype on a broad range of neuropsychological measures in a large sample with adult patients with ADHD and matched healthy controls (*Chapter 2*). Subsequently, the same sample is used to explore gray matter volume differences using structural MRI (*Chapter 3*) and differences in white matter microstructure using DTI (*Chapter 4*). Additionally, presence of comorbidity, a history of medication, gender-

related differences will be investigated. For some neuroanatomical findings, their relation with symptomatology and neuropsychological measures will be examined.

The second specific aim addresses another gap in the literature by focusing on the underlying mechanism of RTV which is currently still unknown. Here the hypothesis that white matter microstructure is related with RTV is examined in the same sample that is used in previous chapters (*Chapter 5*). The last specific aim addresses a gap in the literature about how the *DAT1* gene is associated with ADHD across the lifespan. Here a genetic imaging approach is used that investigating how several *DAT1* variants affect striatal volume. Since it is assumed that the effect of *DAT1* on ADHD is not static throughout the lifespan, the effect of age was examined by including three samples that encompasses children, adolescents and adults diagnosed with ADHD and healthy controls (*Chapter 6*).

Description of study cohorts

For our studies, we used three cohorts: The first cohort (used in *Chapter 2, 3, 4 5 and 6*) is from the Dutch part of the International Multicentre persistent ADHD CollaboraTion (IMpACT - <http://impactadhdgenomics.com> (Franke et al., 2010). Adult patients with ADHD were recruited through the department of Psychiatry of the Radboud university medical center in Nijmegen and through advertisement via the website of 'Balans', a Dutch patient organization for ADHD. In addition, healthy comparison individuals were recruited via advertisements in local newspapers. Participants in this research project were invited for two sessions at the Donders Centre for Cognitive Neuroimaging. The first session consisted of psychiatric interviews and blood withdrawal for DNA analysis. During this first session, patients were interrogated about meeting in- or exclusion criteria. Patients were included if they met DSM-IV-TR criteria for ADHD in childhood as well as adulthood (American Psychiatric Association, 2000). All subjects were assessed using the Diagnostic Interview for Adult ADHD (DIVA) (Kooij, 2010). This interview focuses on the 18 DSM-IV symptoms of ADHD and uses concrete and realistic examples to thoroughly investigate whether the symptom is present now or was in childhood. In order to obtain information about ADHD symptoms and impairment in childhood, additional information is obtained from parents and school reports, whenever possible. Severity of ADHD was assessed by self-report using the ADHD DSM-IV rating scale (Kooij et al., 2005). The Structured Clinical Interview for DSM-IV Criteria (SCID-I&II) (Groenestijn et al., 1999; Weertman et al., 2000) was used for comorbidity assessment and serves as a source to determine our exclusion criteria, i.e. psychosis and addiction in the last 6 months or a current diagnosis of major depression. Additional exclusion criteria were a full-scale IQ estimate less than 70 (assessed using the Wechsler Adult Intelligence Scale-III), neurological disorders, sensorimotor handicaps, non-Caucasian ethnicity and medication use other than psychostimulants or atomoxetine. An extra exclusion criterion for comparison subjects was a current or past neurological or psychiatric disorder according to SCID-I. Assessments were carried out by trained professionals (psychiatrist or psychologists). During the second session, cognitive domains relevant for ADHD were tested using computerized and paper/pencil tasks. Neuroimaging

procedures were also executed on the second testing day. IMpACT-NL is an ongoing research program, which currently contains endophenotype data of over 331 ADHD patients and 155 controls.

The second cohort (used in *Chapter 6*) involved participants of the the NeuroIMAGE study (www.neuroimage.nl) is a follow-up of the Dutch part of the International Multicenter ADHD Genetics (IMAGE) project (Rommelse et al., 2008). It is a multi-site prospective cohort study designed to investigate the course of ADHD, its genetic and environmental determinants, its cognitive and neurobiological underpinnings, and its consequences in adolescence and adulthood. From the original 365 ADHD families and 148 control (CON) IMAGE families, consisting of 506 participants with an ADHD diagnosis, 350 unaffected siblings, and 283 healthy controls, 79 % participated in the NeuroIMAGE follow-up study. Combined with newly recruited participants the NeuroIMAGE study comprehends an assessment of 1,069 children (751 from ADHD families; 318 from CON families) and 848 parents (582 from ADHD families; 266 from CON families). For most families, data for more than one child (82 %) and both parents (82 %) were available. Collected data include a diagnostic interview, behavioural questionnaires, cognitive measures, structural and functional neuroimaging, and genome-wide genetic information (von Rhein et al., 2014)

The third cohort (used in *Chapter 6*) involved 2435 participants of the Brain Imaging Genetics (BIG) study. The BIG study was set up in 2007 as collaboration between the Human Genetics department of the Radboud University Nijmegen Medical Centre and the Donders Centre for Cognitive Neuroimaging of the Radboud University Nijmegen. In 2010, the Max Planck Institute for Psycholinguistics in Nijmegen also joined. In BIG, relations between genes and brain structure and function are investigated in healthy individuals (see for example (Guadalupe et al., 2014)). For this, a continuously growing database has been created with data of structural brain scans and DNA as well as cognitive and behavioral data derived from internet-based testing. The BIG assessment protocol contains the ADHD DSM-IV rating scale (DuPaul et al., 1998), and therefore data of self-reported ADHD symptoms were available, which I studied in conjunction with the neuroimaging data in this population-based study.

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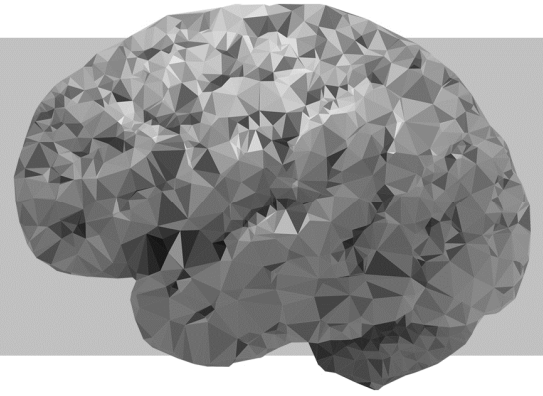
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Cognitive heterogeneity in adult ADHD: A systematic analysis of neuropsychological measurements.

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Abstract

Attention Deficit / Hyperactivity Disorder (ADHD) in childhood is associated with impaired functioning in multiple cognitive domains: executive functioning (EF), reward and timing. Similar impairments have been described for adults with persistent ADHD, but an extensive investigation of neuropsychological functioning in a large sample of adult patients is currently lacking. We systematically examined neuropsychological performance on tasks measuring EF, delay discounting, time estimation and response variability using univariate ANCOVA's comparing patients with persistent ADHD (N = 133, 42% male, mean age 36) and healthy adults (N = 132, 40% male, mean age 36). In addition, we tested which combination of variables provided the highest accuracy in predicting ADHD diagnosis. We also estimated for each individual the severity of neuropsychological dysfunctioning. Lastly, we investigated potential effects of stimulant medication and a history of comorbid major depressive disorder (MDD) on performance. Compared to healthy adults, patients with ADHD showed impaired EF, were more impulsive, and more variable in responding. However, effect sizes were small to moderate (range: 0.05 – 0.70) and 11% of patients did not show neuropsychological dysfunctioning. The best fitting model predicting ADHD included measures from distinct cognitive domains (82.1% specificity, 64.9% sensitivity). Furthermore, patients receiving stimulant medication or with a history of MDD were not distinctively impaired. To conclude, while adults with ADHD as a group are impaired on several cognitive domains, the results confirm that adult ADHD is neuropsychologically heterogeneous. This provides a starting point to investigate individual differences in terms of impaired cognitive pathways.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common and highly heritable neuropsychiatric disorder in childhood that is strongly persistent over time. At least 35% of all childhood patients still meet full ADHD criteria in adulthood (American Psychiatric Association, 2000), and this percentage is much higher (78%) when partial remitted patients are included (Biederman et al., 2010). ADHD has an average prevalence of 2.5 - 4.9 % in the adult population (Simon et al., 2009). The clinical phenotype of ADHD is characterized by persistent, age-inappropriate symptoms of inattention, and / or hyperactivity and impulsivity (American Psychiatric Association, 2000).

ADHD has been associated with neurocognitive dysfunctioning, and over the years, several neuropsychological theories about ADHD etiology have been put forward. One of the most influential theories proposed ADHD to arise from a single core deficit in behavioral inhibition, which leads to secondary impairments in several executive functions (Barkley, 1997). However, this assumption of a central deficit was challenged by data showing that ADHD patients are impaired in multiple neuropsychological domains. It has therefore been proposed that there are distinct pathways to dysfunction, including executive function (EF) deficits, delay aversion, and timing problems (Castellanos et al., 2006; Sonuga-Barke et al., 2010). Although not included in the multiple pathway model, another characteristic of ADHD is performance variability. The inconsistency in performance and the high prevalence of moment-to-moment variability in reaction times is one of the most consistently reported manifestations of ADHD. Reaction time variability (RTV) received extensive discussion as an indicator of cognitive performance, although the exact nature of high RTV in ADHD is still uncertain (Kofler et al., 2013; Tamm et al., 2012).

Studies of cognitive functioning in adults with ADHD suggest that cognitive impairments found in adults resemble those observed in children with ADHD, showing equally moderate effects sizes (for meta-analytic reviews, see (Boonstra et al., 2005; Hervey et al., 2004; Schoechlin and Engel, 2005). Similar results were derived from qualitative reviews (Seidman, 2006; Woods et al., 2002). Recent meta-analyses in adult ADHD focused solely on deficits found in working memory (Alderson et al., 2013) and long-term memory (Skodzik et al., 2013). Furthermore, recent experimental studies on adult ADHD show deficits in attention (Fuermaier et al., 2015; Grane et al., 2014), set-shifting (Boonstra et al., 2010; Hallelund et al., 2012; Rohlf et al., 2012) inhibition (Boonstra et al., 2010; Fuermaier et al., 2015), (working) memory (Fuermaier et al., 2015; Lundervold et al., 2015; Rohlf et al., 2012), delay discounting (Marx et al., 2010), and increased reaction time variability (Feige et al., 2013; Gmehlin et al., 2014; Grane et al., 2014).

From the childhood literature, we know that ADHD is characterized by large heterogeneity at the neuropsychological level, which means that only a minority of ADHD patients shows deficits in each domain and that some patients with ADHD will perform in the normal range (Nigg et al., 2005b). Such heterogeneity was illustrated in a recent study on boys with ADHD (Coghill et al., 2013). Per cognitive domain merely 18-36% of the patients

had an impairment, while 25% of the sample did not show deficient performance in any of the cognitive domains.

Heterogeneity in cognitive performance within a sample of ADHD patients may also arise from differences in medication use or comorbidity. Stimulants are effective for the treatment of clinical symptoms in adult ADHD (Faraone et al., 2004) and also in neuropsychological studies medication is usually seen as a potential moderator. Many neuropsychological studies in ADHD have included patients who had previously taken, or were receiving stimulant medication at the time of the study. To eliminate the acute effects of medication, most studies used a washout period (24h or 48h). However, stimulants may act longer than 48h (McCarthy et al., 2014). Similarly, ADHD patients with a comorbid psychiatric disorder showed greater neuropsychological deficits than ADHD patients without comorbidity (Hervey et al., 2004) and may represent a distinct subgroup, with different cognitive profiles (Fischer et al., 2007). However, it has also been shown that cognitive deficits in adult ADHD cannot be accounted for by comorbid disorders (Nigg et al., 2005a; Silva et al., 2013). Major depressive disorder (MDD) is the most frequently observed comorbidity, and can co-occur with ADHD in up to 50% of the cases (Wilens et al., 2009). MDD has been associated with cognitive difficulties in memory, attention and problem-solving. Only two studies examined comorbid MDD in ADHD to date, both suggesting that current comorbid MDD symptoms may not influence neuropsychological profiles in ADHD (Katz et al., 1998; Riordan et al., 1999). While potential effects of comorbid MDD on cognition are often controlled for by excluding patients with current MDD from a study, many included patients will have remitted MDD. It is currently not known whether adult ADHD patients with MDD in remission are distinctively impaired on cognitive performance, although it has been shown that ADHD symptom severity increases in association with lifetime occurrence of comorbid MDD (Simon et al., 2013).

Reviewing the literature of adult ADHD shows that experimental studies and meta-analyses are limited by relying on relatively small samples with different inclusion criteria and tasks. Those studies had limited power to investigate confounding effects on neuropsychological functioning such as comorbidity or treatment. Also, the investigation of different tasks or functions in different samples has limited the possibility to construct a comprehensive picture of impairments associated with adult ADHD. To improve confidence in the findings, replication/validation in a large cohort of adult ADHD patients is thus desirable. Lastly, except for studies by Seidman et al. (1998), Boonstra et al. (2010), and Fuerrmaier et al. (2015), most studies assessed only a narrow range of neuropsychological tasks. Therefore, we investigated case-control differences on a wide range of well-described neuropsychological tasks using the largest sample of adult ADHD patients to date. Neuropsychological tasks were chosen based on the multiple pathway model and RTV literature described above and measured motor speed, sustained attention, inhibition, delay discounting, time estimation, set-shifting, verbal fluency, working memory, and response variability. We expected effect sizes to be moderate, with strongest effects on RTV as this is a pervasive characteristic observable across tasks (Kofler et al., 2013). Furthermore, we were

interested in the diagnostic relevance of these tasks. From a clinical perspective it is interesting to know the predictive importance of neuropsychological measurements in ADHD classification. Previous literature showed however that neuropsychological measurements have a relatively poor ability to discriminate between children with ADHD and typically developing controls (Sjowall et al., 2013) or adults with ADHD and psychiatric patients without ADHD (Holst and Thorell, 2013). It remains an open question how discriminative the investigated neuropsychological tasks are in a sample of healthy adults with and without ADHD. We further investigated heterogeneity in performance and severity by computing the number of deficient test scores per participant as was previously done in childhood ADHD (Coghill et al., 2013). Additionally, we explored the potential effect of stimulant medication and a history of comorbid MDD on performance.

Experimental procedures

Participants

The study population was the Dutch cohort of the International Multicenter persistent ADHD CollaboraTion (IMpACT - <http://impactadhdgenomics.com> (Franke et al., 2010)). This is an ongoing study that at the time of analysis (1 January 2014) included 298 participants (155 adult ADHD cases, 143 healthy comparison participants). Patients and healthy control participants were recruited at the department of Psychiatry of the Radboud university medical center in Nijmegen and through advertisements. Patients were included if they had previously been diagnosed with persistent ADHD, i.e. present since childhood, by a psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders (4th edition; DSM-IV-TR; (American Psychiatric Association, 2000). Exclusion criteria for participants were psychosis, alcohol or substance addiction in the last six months, current major depression, full-scale IQ estimate <70, neurological disorders, sensorimotor disabilities, non-Caucasian ethnicity, medication use other than psychostimulants, atomoxetine or bupropion and failure to withhold stimulant medication 24 hours prior to testing (see *Procedure* below). Additional exclusion criteria for healthy controls were a current or lifetime neurological or psychiatric disorder in either the proband or his/her first-degree relatives. From the total sample, 33 participants (22 patients, 11 controls) had to be excluded because they met at least one of these exclusion criteria (see Supplementary Table 1).

This study was approved by the regional ethics committee (Centrale Commissie Mensgebonden Onderzoek: CMO Regio Arnhem – Nijmegen; Protocol number III.04.0403). Written informed consent was obtained from all participants.

Procedure

Subjects were invited for two sessions (Supplementary Figure 1), one including a detailed psychiatric assessment and blood withdrawal for biobanking of DNA, RNA and serum. A second session consisted of cognitive testing and neuroimaging procedures. The genetic and neuroimaging data are described elsewhere (i.e. (Franke et al., 2010; Hoogman et al., 2011).

For session 2, participants were requested to withhold stimulant medication 24h prior to testing.

Psychiatric assessment

Both patients and controls were assessed using the structured Diagnostic Interview for ADHD in Adults (DIVA, (Kooij, 2010)). This interview focuses on the 18 DSM-IV symptoms of ADHD and uses concrete and realistic examples to thoroughly investigate whether a symptom is currently present or was present in childhood. In addition, a self-report questionnaire on current symptoms was obtained using the ADHD Rating Scale-IV (Kooij et al., 2005). The Dutch version of the Structured Clinical Interview for DSM-IV, SCID-I and SCID-II (Groenestijn et al., 1999; Weertman et al., 2000) was used to identify lifetime Axis I and II disorders. Twenty-two patients and 12 controls did not participate in the clinical interview. These participants were included in the main analysis based on a prior diagnosis of ADHD by a psychiatrist and if they reached clinical threshold for ADHD based on the self-report scale. They were excluded from the analysis of comorbidity (see below).

Neuropsychological measurements

The neuropsychological test battery included measures tapping into EF (working memory, attention, inhibition, set-shifting, verbal fluency), delay discounting, and time estimation. Details about tasks and main outcome measures are described in Table 1 and the supplementary text. To estimate IQ, Vocabulary and Block Design of the Wechsler Adult Intelligence Scale (WAIS-III) were administered (Wechsler, 1997). The tests were always administered in the same order.

Table 1. Tasks and outcome measures of the neuropsychological test battery

Task	Task description*	Cognitive domain	Outcome measure
1. Baseline speed task	Participants respond with a button press as quickly as possible when a fixation cross changes into a block-shape	Motor speed & reaction time	Mean RT SD of RT
2. WAIS-III Digit span task	Participants repeat strings of digits that are read aloud by the experimenter. In the backward condition, strings are repeated in reverse order. Each trial the working memory load increases.	Executive functioning: Working memory	Forward digit span score Backward digit span score
3. Flanker task	Participants respond with a button press to the color of the center block (yellow or blue), flanked by other blocks. In part 1, the center block is flanked by blocks of the same color (congruent trial) or a different color (green, neutral trial). In part 2, the neutral trials are replaced by incongruent trials (flanking blocks with the color of the alternative response).	Executive functioning: Inhibition	Total mean RT (average over part 1 and 2) Total SD of RT (average over part 1 and 2) Inhibition RT (difference in RT on congruent and incongruent trials in part 2) Inhibition errors (difference in error rate between congruent and incongruent trials in part 2)

4. Sustained attention dots task (SA-dots)	Three, four or five dots are presented on the screen. Participants respond with a button press with the dominant hand to four dots and with the non-dominant hand to three or five dots. An erroneous button press to three or five dots is a false alarm; an erroneous button press to four dots is a miss. For analysis, the task is split up into ten blocks, or series, in order to compute variance in performance over time. The duration of task is 20 minutes.	Executive functioning: Attention & inhibition	Mean series completion time SD series completion time SD series errors (SD of the errors made across blocks) Response bias (the difference between the number of misses and the number of false alarms across the entire task)
5. Sustained Attention to Response Task (SART)	Go/No-Go task. Participants respond with a button press to single digits presented on the screen (1 -9), but to withhold a response when the digit 3 is presented.	Executive functioning: Attention & inhibition	Number of commission errors Number of omission errors Mean RT hits SD of RT hits
6. Trailmaking task	Participants need to connect dots containing numbers in consecutive order (part A) or alternating between numbers and letters in consecutive order (part B).	Executive functioning: Motor control & set-shifting	Time to complete part A Time to complete part B Difference in time to complete part B and time to complete part A
7. Semantic category and initial letter fluency	Participants name as many animals or professions they can think of in one minute. Next, they name as many words starting with a 'D', 'A' or 'T' as they can think of in one minute.	Executive functioning: Verbal fluency	Number of words mentioned in category animals Number of words mentioned in category professions Number of words mentioned in category letters (total of 3 letter-trials)
8. Delay discounting task	Participants repeatedly have to choose between two hypothetical incentives that differ in the value (money) and delay (time until the money would be received). The impulsivity parameter (k) is computed from the present value of the delayed reward (V), the real value of the delayed reward (a) and the delay in days (D) with the formula: $V = a / (1 + kD)$.	Delay aversion & impulsivity	K 100 (impulsivity high rewards) K 30 (impulsivity intermediate rewards) K 10 (impulsivity low rewards)
9. Time estimation task	Participants have to respond with a button press exactly one second after hearing a sound beep. First, during a training session the length of a second is shown several times. During the experiment, feedback is given ('too slow', 'correct', 'too fast').	Timing	Median response time Absolute deviation of the median response time from 1000 ms

RT = reaction time; SD = standard deviation; ms = milliseconds.

* More detailed information about the tasks, including references, can be found in the Supplementary materials.

Data analysis of neuropsychological tasks

All measures were entered as raw scores in the analyses. Performance on each neuropsychological measure was entered as the dependent variable in separate univariate ANCOVA's, testing the difference between patients and controls. Age and gender were entered as covariates of no interest in order to reduce error variance (Miller and Chapman, 2001). This was justified as age and gender did not differ between the groups. We therefore also did not investigate interactions between diagnosis and age or gender. As IQ is correlated with performance on many neuropsychological tasks, we investigated whether adding estimated IQ as an additional covariate would influence the findings. As IQ also did not differ between groups, this analysis using ANCOVA was justified and did not serve to control for IQ. Assumptions with respect to the residuals were checked and neuropsychological measures were transformed if necessary. Outliers were defined as having a score more extreme than four times the standard deviation above or below the mean per group (Leth-Steensen et al., 2000; Nigg et al., 2005a). This threshold guarded against artifacts and chance level performance, while still including cases performing at the extreme of the normal distribution. If a participant's score was an outlier on one outcome variable of a task, his/her scores on all outcome variables from that task were excluded. Effect sizes were computed as Cohen's D, using the corrected means from the ANCOVA's (Cohen, 1988).

Multiple comparison correction was performed by estimating the effective number of independent tests (Meff) (Li and Ji, 2005). This method takes into account the correlation structure between measures and calculates the Meff based on the observed eigenvalue variance of the different neuropsychological measures using the matSpD interface (<http://genepi.qimr.edu.au/general/daleN/matSpD/>). The p -value for significance was determined as 0.05 divided by Meff. Twenty-seven measures resulted in twenty-two independent tests and therefore, only effects with a p -value < 0.0023 were considered significant.

Second, to investigate discriminating ability of the neuropsychological test battery, we used a step-wise backward logistic regression model. To maximize power, with our sample size, we included only those neuropsychological measures that were nominally significant in the case-control comparison to determine the model with the highest prediction accuracy of diagnostic status. Variables were retained in the model when they significantly contributed to the likelihood ratio statistic, all other variables were excluded.

To investigate heterogeneity in cognitive impairments, we computed the number of deficient test scores for each participant. Similar to previous studies, a deficient score was defined as performance below the 10th percentile of the performance distribution of the control group (Coghill et al., 2013; Nigg et al., 2005b). For variables where higher scores indicated worse performance, deficiency was defined as a score above the 90th percentile of performance distribution of the control group. For the variable 'time estimation median response time' performance at both lower and upper extreme was scored as deficient. As not all participants had completed data for all tasks, we computed the relative number of

deficient test scores as a percentage of the total number of scores for that participant. We labeled between 1% and 20% deficient test scores as 'mildly impaired', between 20% and 40% as 'impaired' and above 40% as 'severely impaired'. The difference between cases and controls in the number of relative deficient test scores was computed using an ANCOVA with age and gender as covariates. In addition, we repeated the same analysis in a restricted group of only those participants with complete data (N = 168).

Effects of stimulant medication and history of MDD

We conducted two exploratory analyses. First, in order to investigate stimulant medication effects on neuropsychological measures, we used separate ANCOVA's for each neuropsychological measure comparing medication naïve patients (N = 20), medicated patients (N = 83), and healthy control participants (N = 132), with age and gender as covariates. Second, we conducted a similar analysis comparing patients with at least one lifetime MDD episode (now in remission, N = 55), patients without a history of MDD (N = 68), and healthy controls without prior episodes of MDD (N = 112). Twenty healthy control participants reported to have experienced depressive episodes in the past and were therefore excluded from this analysis. For both analyses, in the case of a main effect of group on the neuropsychological measure, we tested post-hoc the differences between groups. These post-hoc tests were Bonferroni corrected for multiple testing.

Results

Demographics

A total of 265 participants (132 healthy controls and 133 ADHD patients) were included in the analyses. Demographic information is provided in Table 2. Patients and controls did not differ in age, handedness, and estimated IQ. Gender was equally distributed across groups. Patients had received fewer years of education than controls. As expected, patients had significantly more ADHD symptoms based on the diagnostic interview and self-report. Information about psychiatric comorbidities and medication is summarized in Supplementary Tables 2 and 3.

Table 2. Demographics (N = 265^a)

	Healthy controls (N = 132)	ADHD patients (N = 133)	p-value
Gender	53 (40.2%) male	56 (42.1%) male	n.s.
Age	36.30 (11.75), range 19-63	35.56 (10.40), range 18 - 59	n.s.
Estimated IQ ^b	109.97 (14.90)	107.83 (14.28)	n.s.
Education ^c	5.16 (0.81), range 3 - 7	4.70 (0.80), range 2 - 7	< .001
Repeated school years (once or more)	53 (40.2%)	77 (57.9%)	.005
Non-completed education programs (one or more)	40 (33.3%) (N = 128)	87 (67.4%) (N = 129)	< .001
Handedness	115 (87.1 %) right, 13 (9.8%) left, 3 (2.3%) ambidextrous (N = 131)	113 (85%) right, 16 (12%) left, 4 (3%) ambidextrous	n.s.
Inattentive symptoms (DIVA)	0.39 (0.83), 0 – 4 (N = 120)	7.38 (1.55), 3 – 9 (N = 112)	< .001
Hyperactive / impulsive symptoms (DIVA)	0.52 (0.98), 0 – 4 (N = 120)	5.76 (2.27), 0 – 9 (N = 112)	< .001
Total symptoms (DIVA)	0.91 (1.43), 0 – 8 (N = 120)	13.14 (2.76), 7 – 18 (N = 112)	< .001
Inattentive symptoms (selfreport)	0.53 (0.98), 0 – 5 (N = 131)	6.40 (2.09), 0 – 9	< .001
Hyperactive / impulsive symptoms (selfreport)	0.89 (1.44), 0 – 6 (N = 131)	5.58 (2.26), 0 - 9	< .001
Total symptoms (selfreport)	1.42 (2.14), 0 – 9 (N = 131)	11.98 (3.37), 1 - 18	< .001

Data show as: mean (standard deviation), minimum – maximum. P-values represent the significance of the group difference, tested with independent samples t-tests for continuous data or Pearson Chi-square tests for categorical data.

^a 32 subjects from the total sample were excluded from analyses according to our exclusion criteria.

^b IQ was estimated based on performance on the WAIS-III block pattern and vocabulary tasks.

^c Education level was coded from 1 (unfinished primary school) to 7 (post-university).

^d DIVA interview data was missing for 22 patients.

Effect of diagnosis on cognitive performance

Findings from the case-control comparison of neuropsychological performance are summarized in Table 3. In the domain of EF patients were impaired on working memory and attention, but no group differences were found for inhibition (Flanker and SART task) and verbal fluency. In the domain of delay aversion patients performed worse than controls on the delay discounting task, but not in the domain of timing (time estimation task). Across several tasks, patients were also more variable in their reaction times than controls. Response speed did not differ between patients and controls in most tasks, except for both conditions of the Trailmaking tasks. Effect sizes were in the small to medium range, with the largest effect on the SA-dots task where patients showed more fluctuation in errors across blocks (effect size = -0.71). Adding the covariate IQ, in addition to age and gender, did not significantly alter the results.

Table 3. Case/control analysis of cognitive performance (N = 265)

NPO Task	Variable	Healthy Controls Mean (SD)	ADHD Mean (SD)	ANCOVA F (df), <i>p</i> -value	Effect size Cohen's D
1. Digit span test HC: N =132 ADHD: N = 128	Forward score	9.83 (2.36)	8.99 (1.95)	10.45 (1, 256), <i>p</i> = .001*	0.40
	Backward score	7.49 (2.34)	6.69 (2.22)	8.62 (1,256), <i>p</i> = .004	0.37
2. Baseline speed (ANT) HC: N =130 ADHD: N = 129	Mean RT	313.28 (49.42)	316.83 (55.29)	0.40 (1, 255), n.s.	-0.07
	SD of RT†	4.08 (0.53)	4.26 (0.57)	7.47 (1,255), <i>p</i> = .007	-0.34
3. Flanker task (ANT) HC: N =127 ADHD: N = 123	Total mean RT	525.37 (73.00)	537.93 (92.87)	1.85 (1, 246), n.s.	-0.18
	Total SD of RT	93.74 (37.04)	118.39 (58.87)	15.90 (1, 246), <i>p</i> < .001*	-0.51
	Inhibition RT	28.44 (28.25)	23.13 (40.76)	1.45 (1, 246), n.s.	0.14
	Inhibition errors	0.68 (1.48)	0.63 (1.47)	0.11 (1, 246), n.s.	0.05
4. SAdots (ANT) HC: N =128 ADHD: N = 123	Mean series completion time	899.05 (129.21)	944.71 (186.08)	5.03 (1,247), <i>P</i> = .026	-0.28
	SD completion time†	3.81 (0.44)	4.07 (0.53)	16.82 (1, 247), <i>p</i> < .001*	-0.52
	SD errors†	0.70 (0.19)	0.86 (0.26)	32.03 (1,247), <i>p</i> < .001*	-0.71
	Response bias	5.05 (6.10)	9.16 (9.51)	18.35 (1,247), <i>p</i> < .001*	-0.52
5. SART HC: N = 110 ADHD: N = 104	Commission errors	9.31 (5.03)	10.51 (4.87)	3.02 (1,210), n.s.	-0.25
	Omission errors	2.63 (3.57)	4.04 (4.97)	5.72 (1,210), <i>p</i> = .018	-0.32
	Mean RT hits	315.50 (57.48)	326.09 (60.74)	2.44 (1,210), n.s.	-0.21
	SD or RT†	4.35 (0.36)	4.56 (0.44)	14.17 (1,210), <i>p</i> < .001*	-0.53
6. Fluency HC: N = 132 ADHD: N = 131	Category; Animals	27.76 (5.77)	25.85 (5.97)	6.84 (1,259), <i>p</i> = .009	0.32
	Category: professions	20.27 (5.23)	19.81 (5.09)	0.48 (1,259), n.s.	0.10
	Letters	41.91 (10.51)	38.95 (10.87)	5.15 (1,259), <i>p</i> = .024	0.29
7. Time estimation HC: N = 126 ADHD: N = 116	Median response time	1007.09 (67.61)	997.94 (82.21)	1.13 (1,238), n.s.	0.14
	Absolute deviation of the median response time from 1000 ms	49.38 (46.38)	63.92 (51.40)	5.49 (1,238), <i>p</i> = .020	-0.30
8. Delay Discounting HC: N = 123 ADHD: N = 109	K 100†	-5.25 (1.54)	-4.50 (1.65)	12.85 (1,228), <i>p</i> < .001*	-0.48
	K 30†	-4.76 (1.65)	-4.38 (1.66)	3.15(1,228), n.s.	-0.23
	K 10†	-4.39 (1.43)	-3.97 (1.67)	4.43(1,228), <i>p</i> = .036	-0.27
9. Trailmaking	Part A	23.70 (7.51)	26.80 (8.24)	11.60 (1,256),	-0.43

task				$p = .001^*$	
HC: N = 132	Part B	50.06 (17.30)	57.89 (20.30)	12.50 (1,254),	-0.44
ADHD: N = 128				$p < .001^*$	
	Part B - A	26.33 (13.51)	31.00 (18.38)	5.88 (1,254),	-0.30
				$p = .016$	

ANCOVA testing the effect of group for each neuropsychological measure, with age and gender as covariates.

† log-transformed variable to a normal distribution.

* indicates p -values surviving correction for the effective number of independent tests conducted ($N = 22$, significance threshold (type 1 error rate at 5%) = 0.0023)(Li and Ji, 2005).

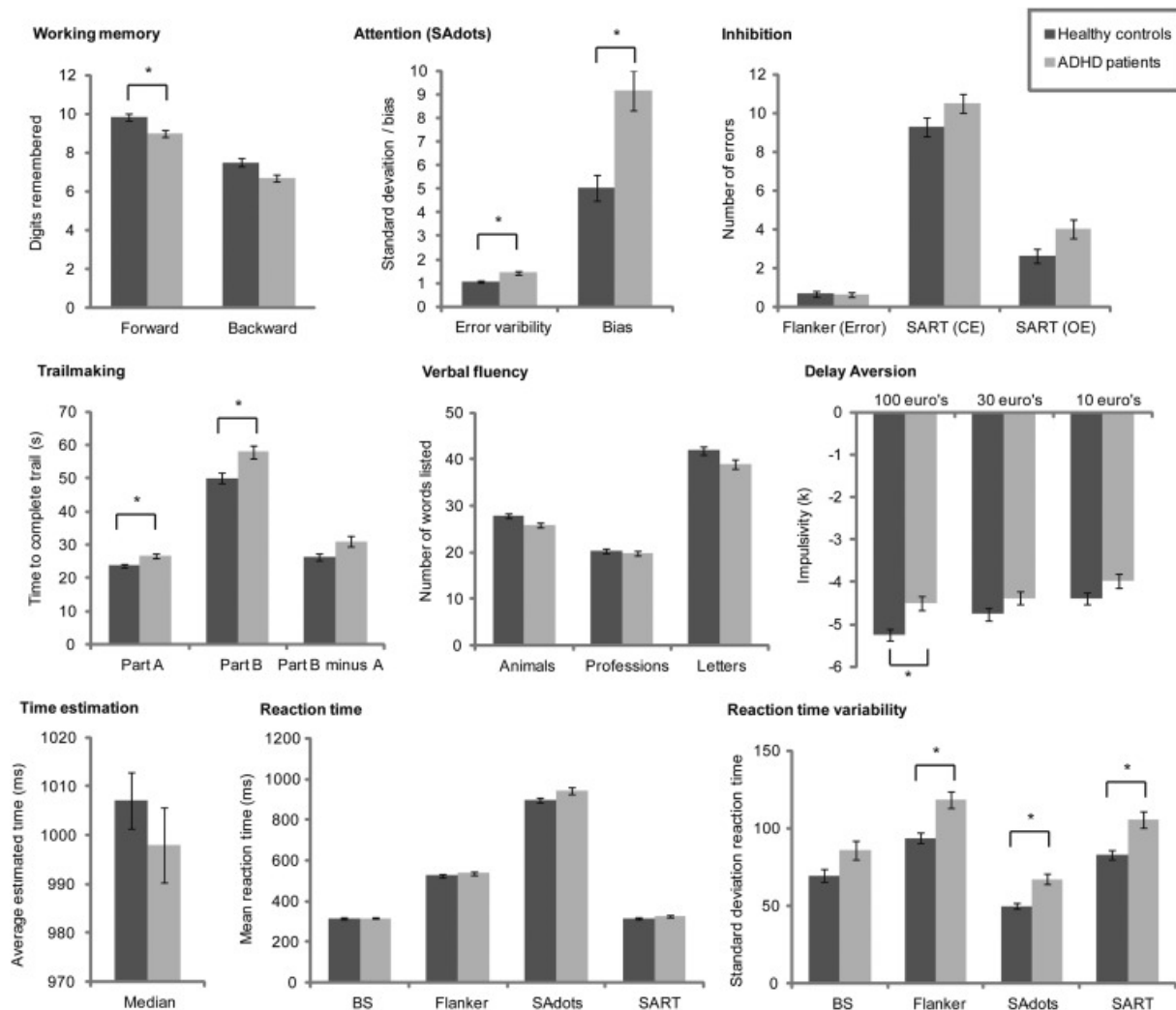


Figure 1. Differences in performance between ADHD patients and controls on measurements from several cognitive domains. Bar graphs indicate the average performance per group for each neuropsychological measure (time estimation absolute median deviation from 1000 ms is not shown); error bars represent the standard error of the mean. Dark grey bars represent the healthy control group, lighter grey bars represent ADHD patient group. An asterix (*) indicates measures where patients differed significantly from controls.

Variables predicting ADHD diagnosis

A stepwise backwards logistic regression identified six out of 17 variables to significantly contribute to a model predicting diagnosis: Digit span (forward), Flanker (total SD of RT), SAdots (SD series errors and response bias), Delay discounting (k100) and Time estimation (absolute median deviation from 1000ms). The entire model significantly distinguished patients from controls (Log-likelihood = 174.13, R^2 (Nagelkerke) = 0.39, $\chi^2 = 57.54$ (6 df), $p < .001$).

0.001) and had a sensitivity (correctly predicting patients) of 64.9% and a specificity (correctly predicting controls) of 82.1%. Model details are shown in Supplementary Table 4.

Number of deficient test scores across all outcome measures

Patients had deficient test scores on a significant larger proportion of variables than controls (mean controls = 9.16% (SD = 9.23), mean ADHD = 15.82% (SD = 13.55), $F = 22.34$ (1,261), $p < 0.001$). This effect remained when only including participants with complete data ($F = 13.08$ (1,164), $p < 0.001$). As apparent from Figure 2, there was a large variability between individual patients, with some patients not having any deficient scores (11%), while others were severely impaired (5%). The majority (62%) of patients was mildly impaired, and 23% was impaired. This variability was also present in the control group, although here the majority of participants (64%) had deficient scores on 10% or less of the outcome variables.

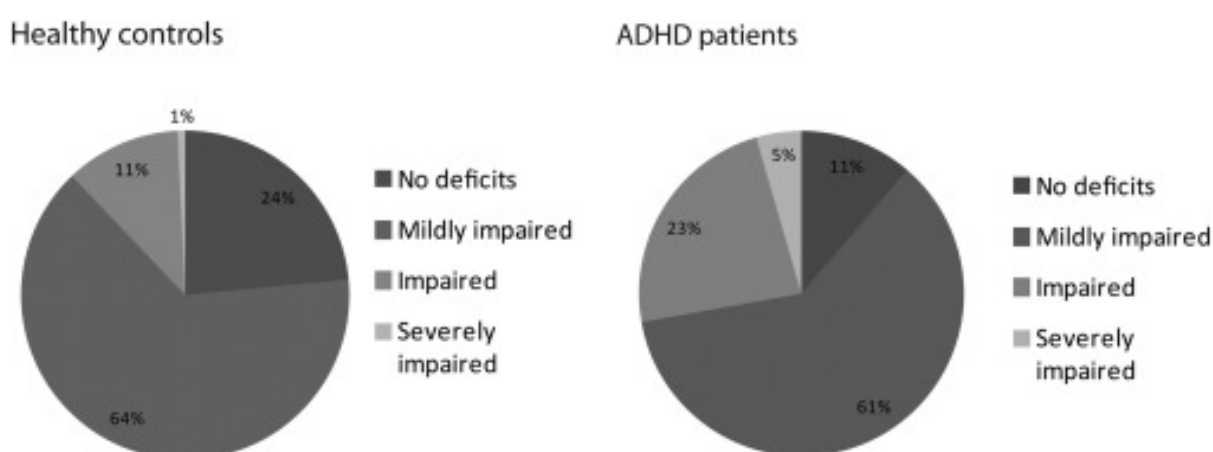


Figure 2. Deficient test scores across participants. For each participant, deficient test scores were computed as the number of test scores that were below the score of the bottom 10% of the control group, divided by the total number of test scores of that participant and multiplied with 100%. The sections indicate the percentage of participants that had a deficient test score within a certain bin. ‘Mildly impaired’ are participants with 1–20% deficient test scores, ‘impaired’ are those with 20–40% deficient test scores and those with more than 40% deficient were labeled as ‘severely impaired’.

Effects of stimulant medication and history of MDD on neuropsychological measures

We additionally investigated the effect of stimulant medication on neuropsychological performance by comparing medication naïve patients, medicated patients and controls. Group effects were all in the same direction as in the main case-control analysis, although smaller (Supplementary Table 5). Post-hoc comparisons indicated that on the time estimation task medication naïve patients responded faster than medicated patients and controls.

The main effects from the case-control analyses were also reproduced when comparing healthy controls to ADHD patients with and without a history of MDD (Supplementary Table 6). On none of the neuropsychological measures did patients with a history of MDD differ from patients without this comorbidity. However, on several measures patients with a history of MDD did not differ from controls and patients without MDD, despite a main effect of group.

Discussion

In this study we examined the neuropsychological performance of a large group of patients with persistent ADHD and healthy adult control participants on a broad range of neuropsychological tasks. As a group, patients with ADHD showed impaired EF, especially working memory and sustained attention, were more sensitive to delay aversion, and had increased response variability as compared to healthy controls. Stepwise logistic regression analysis showed that measures from distinct cognitive domains collectively contributed to the predictive model explaining variance in ADHD. Despite this, the model had limited predictive power for diagnostic status. Cognitive heterogeneity of the sample was also apparent from large inter-individual variability in the number of deficient test scores, especially in the ADHD group, but also in controls. Strikingly, no case-control differences were found in tasks measuring inhibition and timing in our test battery. Effect sizes were small to moderate, and medication and a history of MDD comorbidity did not explain differences in performance in adult ADHD.

As described, a popular model of childhood ADHD implicates three neuropsychological pathways in childhood ADHD, one involving EF deficits, one involving altered reward processing, and one involving temporal processing deficits (Castellanos et al., 2006; Sonuga-Barke et al., 2010). We report evidence for impairment in EF and reward processing, but not in temporal processing in patients with persistent ADHD. Our finding that EF deficits are primarily related to working memory and sustained attention is in agreement with the adult ADHD literature (Boonstra et al., 2005; Hervey et al., 2004). This result stresses the significance of attentional problems in adult ADHD and may reflect the fact that the symptoms of hyperactivity and impulsivity decrease as ADHD children approach adulthood (Biederman et al., 2000). It has been suggested that IQ may play a role in explaining working memory and attention deficits in adult ADHD (Boonstra et al., 2010; Murphy et al., 2001). However, as the groups did not differ on IQ, and covarying for IQ did not alter the results, this explanation is unlikely.

Contrary to expectations, we did not find EF differences related to inhibition, set-shifting, or verbal fluency. The ability to inhibit a response has been posited as a core domain impaired in ADHD (Barkley, 1997), and has been found in several studies of adult ADHD (Boonstra et al., 2010; Boonstra et al., 2005; Hervey et al., 2004), though not in others (Gmehlin et al., 2014; Hallelund et al., 2012). We used the SART and Flanker tasks to measure inhibition, but are cautious to interpret our null findings as strong evidence against inhibition deficits in adult ADHD. First, the Flanker task showed a ceiling effect in inhibition errors, which is consistent with findings in early adolescence (Drechsler et al., 2005; Harms et al., 2014). This task may therefore lack sensitivity to measure inhibition impairments in adult ADHD. Second, on the SART, the number of commission errors (measuring inhibition) did not differ between patients and controls, nor did the number of omission errors (measuring attention). To better characterize inhibition deficits in adult ADHD, a more sensitive measure would be the stop signal reaction time as measured with a stop signal task. Such a task is unique in that it has variable inter-stimulus intervals often at a rapid pace

that requires participants to interrupt an already ongoing response. This task design may provoke impulsive responses among participants more strongly and may thus be more sensitive to inhibition problems in ADHD (Epstein et al., 2001). Indeed, manipulation of response prepotency was effective in evoking response inhibition difficulties in adult ADHD patients (Grane et al., 2014).

Set-shifting is another component of EF, which we measured using part B of the Trailmaking task. Even though patients were slower on this part of the task, they were equally slow on part A, which measures motor speed (Nigg et al., 2005a). This finding is in line with other studies in adult ADHD suggesting that deficits in set-shifting are explained by impaired processing speed (Rohlf et al., 2012). We thus conclude that set-shifting as measured with the Trailmaking task was not impaired in the adult ADHD group. Lastly, patients did not differ from controls in verbal fluency measures, which contradicts previous findings (Boonstra et al., 2005; Hervey et al., 2004). This could be due to the good IQ-matching between patients and controls in our sample, whereas in other studies patients had lower IQ than controls. In children it was found that IQ significantly correlated with verbal fluency (Ardila et al., 2000). Hence, previously reported differences in verbal fluency may be more attributable to differences in IQ than to ADHD.

Delay aversion may represent a second neuropsychological pathway towards ADHD, linked to altered processing of rewards (Sonuga-Barke, 2002). Our results of stronger delay discounting in patients are in line with other evidence of increased impulsive decision making in persistent ADHD (Marx et al., 2010; Paloyelis et al., 2009). The tendency to prefer immediate (smaller) over delayed (larger) rewards is also considered to be an aspect of impulsivity potentially important for the development of substance use disorders (Dick et al., 2010). Therefore, stronger delay aversion might represent a vulnerability marker for substance abuse in ADHD (Bickel et al., 2012). A third pathway involves temporal processing deficits (Sonuga-Barke et al., 2010). In the present study, patients did not differ from controls on timing accuracy using a time estimation task with an interval of one second. These findings are supported by a recent study using the same task, which showed deficits in time estimation accuracy were present in adolescents with ADHD, but not in adults (Thissen et al., 2014). However, another study, which examined time estimation in adults with ADHD using several time intervals (2, 6, 12, 24, 36 and 48 seconds), found that the patients produced errors predominantly at interval durations of 36 and 48 seconds (Marx et al., 2010). This may suggest that tasks using an interval of one second may not be sensitive enough to measure existing timing deficits in adult ADHD.

In the analyses comparing patients and controls the largest effect sizes were observed for measures of performance variability, both in terms of fluctuations in errors as in reaction times. This confirms our hypothesis, which was based on previous studies identifying RTV as one of the most robust features of ADHD (Kofler et al., 2013; Tamm et al., 2012). Notably, the average reaction time on the tasks used to measure RTV did not differ between patients and controls, supporting the notion that RTV is not attributable to differences in processing speed (Kofler et al., 2013). Rather, RTV is thought to reflect lapses

in attention that produce a skewed reaction time distribution with a large tail (Leth-Steensen et al., 2000). More thorough investigation of RTV used ex-Gaussian modeling and showed that increased RTV is partly due to overly slow responses (Feige et al., 2013; Gmehlin et al., 2014; Wolfers et al., 2015). These slow responses are reflected by the ex-Gaussian parameter *tau*, which represents the exponential component of the reaction time distribution. Recently, we showed that the *tau* parameter was associated with the microstructural integrity of the right superior longitudinal fasciculus, a white matter tract implicated in both attention and ADHD (Wolfers et al., 2015). Taken together, such findings suggest a neurobiological basis for within-subject variability in ADHD. Interestingly, we observed the largest effect size for the variance in errors made during the SA-dots task. This is a promising novel measure for future studies on sustained attention in ADHD using a continuous performance task.

We achieved limited accuracy in predicting ADHD diagnosis from neuropsychological performance, despite the large number of cognitive test variables available. This is consistent with what was previously found in children with ADHD (Sjowall et al., 2013). The best fitting predictive model included six measures from different cognitive domains (EF, response variability, timing and delay aversion) and reached 82.1% specificity and 64.9% sensitivity. This rather low sensitivity makes a test based on cognitive measures insufficient as a diagnostic tool for ADHD in clinical practice. The variables retained in the final model of the logistic regression could be influenced by outliers, as these can be expected to contribute strongly to the model. However, all extreme outliers were removed from the data before data analysis, reducing the effect of erroneous data on the model. Rather, the variables in the model are likely to be most sensitive to behavioral impairments associated with ADHD, as was also reflected in the effect sizes of most of these variables in the case-control analysis. Importantly, measures from distinct cognitive domains collectively contributed to the model, indicating that there is not a single cognitive task or domain sufficient for explaining ADHD on the group level. This is in agreement with the theory of multiple pathways leading to impairment in ADHD (Sonuga-Barke et al., 2010). Besides that heterogeneity can be explained by impairments in multiple cognitive pathways, we also observed differences in severity of impairments between individuals. The majority of patients were impaired on less than 20% of all cognitive measures, and while a small proportion of patients had more than 40% deficient test scores, 11% of patients did not show any deficit. This is in line with studies in childhood ADHD (Coghill et al., 2013; Nigg et al., 2005b; Sonuga-Barke et al., 2010). Importantly, only 23% of our healthy control participants did not show any deficits, which is much lower than the previously reported 53% and 60% (Coghill et al., 2013; Nigg et al., 2005b). However, these differences between studies can be explained by the fact that the current study included many more variables (27 instead of four and six). Furthermore, the majority of controls fell in the ‘mildly impaired’ group, which means they performed deficiently on 1-20% of the tasks. Seeing that the criterion for having a deficient test score was performing at the extreme of the control distribution, it would be expected that controls perform deficiently on some tasks.

The current findings provide a starting point to investigate individual differences in terms of impaired cognitive pathways, for instance by using clustering analyses on the neuropsychological data (Fair et al., 2012). Such an approach follows the recently proposed strategy by the NIMH, called Research Domain Criteria (RDoC) to investigate mental disorders in a dimensional instead of categorical manner (<http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml>). Neurocognitive measures can be used to characterize psychopathology without being restricted to current disorder categories. This will aid in the understanding of the neurobiological and behavioral underpinnings of mental disorders. Furthermore, neuropsychological investigations may be helpful for clinicians in characterizing individual differences, allowing more personalized treatments.

We did not find evidence for subgroups within the patient group, neither due to stimulant medication treatment nor history of comorbid MDD, which could explain the observed cognitive heterogeneity. Medication use did not influence task performance in our exploratory analysis; medication naïve patients performed similar to medicated patients. Mechanisms linking pharmacological actions of stimulants to neuropsychological processes are speculative, although our results support observations that, in adult ADHD, stimulants seem to produce little improvement on a variety of neuropsychological tasks (Advokat, 2010; Turner et al., 2005). Similarly, the group of patients with a comorbidity in the form of a history of MDD did not seem to differ greatly from the group without this comorbidity in terms of neuropsychological functioning. This extends earlier findings and suggest that ADHD patients diagnosed with current or remitted MDD show similar neuropsychological profiles as patients diagnosed with ADHD alone (Katz et al., 1998; Riordan et al., 1999). It should be noted however that this study was not set up to investigate the effects of stimulant medication or differences between patients with and without a history of comorbid MDD, hence these effects should be investigate further.

The findings presented here should be considered in light of several strengths and weaknesses. This study is unique in its large, well-defined naturalistic sample of patients and a well-matched control sample. We have used a large battery of tasks covering EF, timing, and delay aversion domains. This allows our findings to be interpreted on the scale of cognitive domains instead of on a task-specific level. Our sample was large enough to investigate effects of (at least one) comorbidity. However, our investigation of the effect of stimulant medication was likely underpowered as there were only 20 medication naïve patients in our sample. Investigating the effects of stimulant medication in adults is challenging, as by definition these patients have been symptomatic for a long period. It would therefore be more relevant to investigate the effect of medication duration across patients, but this requires well-documented medication use history, which was not available. Additionally, our findings are limited by the tasks included in our testing battery. We did not include measures tapping into the domains of planning or decision making, which are also important in ADHD psychopathology. Furthermore, our measures of time estimation could be improved by having longer timing intervals. Similarly, inhibition could be measured by

computing stop-signal reaction times from a stop signal task. Including such measures might improve the predictive power for diagnostic status.

To conclude, our study provides novel insights into adult ADHD neuropsychology as well as confirmation of findings observed in earlier, smaller studies. In summary, our study adds to the literature in the following ways: 1) compared with previous studies, our sample size is almost two (Seidman et al., 1998) or three times larger (Boonstra et al., 2010; Fuermaier et al., 2015); 2) we also examined delay aversion and timing deficits which was not sufficiently covered by previous work; 3) while other studies investigated variability in reaction times only, we also investigated variability in errors made during a continuous performance task; 4) we investigated confounding effects of depression history and stimulant treatment (the ADHD patient samples from Seidman et al. (1998) and Boonstra et al. (2010) were all medication-naïve); 5) ours was the first study in adult ADHD to calculate the number of deficient test scores per participant as was previously done in childhood ADHD (Coghill et al., 2013); 6) we studied not only simple group differences but also measures of sensitivity and specificity to examine the discriminatory ability of the neuropsychological test battery in adult ADHD. Our comprehensive analysis of cognitive performance in a large sample of patients with persistent ADHD and well-matched healthy control participants confirms that several cognitive domains are affected in the adult ADHD population, with moderate effect sizes. Both the ADHD and the control sample were heterogeneous in their cognitive performance, with large differences in the number of tasks on which participants scored deficient. In line with this, a predictive model including measures from several domains had limited power to predict diagnostic status. Neuropsychological tasks may therefore be more relevant for characterizing individual impairments that can specifically be targeted with personalized treatment. Future studies focusing on inter-individual differences in performance of patients may aid in a better understanding of ADHD etiology and its persistence, also in terms of the underlying biology.

Supplementary information

Description of neuropsychological tasks

Baseline speed task: A fixation cross is shown on a computer screen, which in variable intervals changes into a block. The participant is asked to react as fast as possible to this block by pressing a key. Both the non-dominant and dominant hand are assessed. Scores are averaged over dominant and non-dominant hand. This task is part of the ANT testing battery (De Sonneville, 1999; Huijbregts et al., 2002).

Digit span task: Strings of digits are read aloud by the experimenter. In the forward condition the participant is asked to repeat the string of digits in the same order. In the backward condition the participant is asked to repeat the digits in the reverse order. On each trial the number of digits to be remembered increases. When errors are made on two consecutive trials, the experiment stops. This task is part of the WAIS-III (Wechsler, 1997).

Flanker task: The participant is presented a matrix of nine blocks (3 x 3) and has to respond by indicating if the color of the middle block is blue or yellow (left or right button press). In part 1 of the task, this block is flanked by other blocks in the same color as is the middle block (congruent trial), or in a different color (neutral trial, green blocks). In part 2, the middle block is flanked by blocks of the same color (congruent trial), or by blocks that have the color of the alternative response (incongruent trial), for example, a yellow block flanked by blue blocks or vice versa. This task is part of the ANT testing battery (De Sonneville, 1999; Huijbregts et al., 2002).

Sustained attention dots task (SAdots): Participants are asked to react to a series of dots on the screen; there can be three, four or five dots presented simultaneously. Dots appear in a random order in a paced tempo. When three or five dots appear on the screen, the participant has to react with, the 'no-key' (the key handled by the non-dominant hand) and when four dots appear the participant is asked to react, as quickly as possible, with the 'yes-key' (the key handled by the dominant hand). Pressing the 'no-key' when 4 dots appear is called a false alarm. Pressing the 'yes-key' when three or five dots appear is called a miss. For analysis, the task is split up into 10 blocks, or series, in order to compute variance in performance over time. For each of the 10 blocks, accuracy was calculated by the number of misses and the number of false alarms. Fluctuation in errors across blocks was then calculated using the within-subject standard deviation of errors. This task is part of the ANT testing battery (De Sonneville, 1999; Huijbregts et al., 2008).

Sustained attention to response task (SART): This task is an adaptation of the Go/NoGo task. A stream of digits (ranging from 1 to 9) is presented on the screen. The participant is asked to react to these as quickly as possible by pressing a button on a buttonbox. The stimuli ensure that reactions follow a certain pace. When the digit 3 is presented, the participant has to withhold a response. A commission error is made when the participant presses the

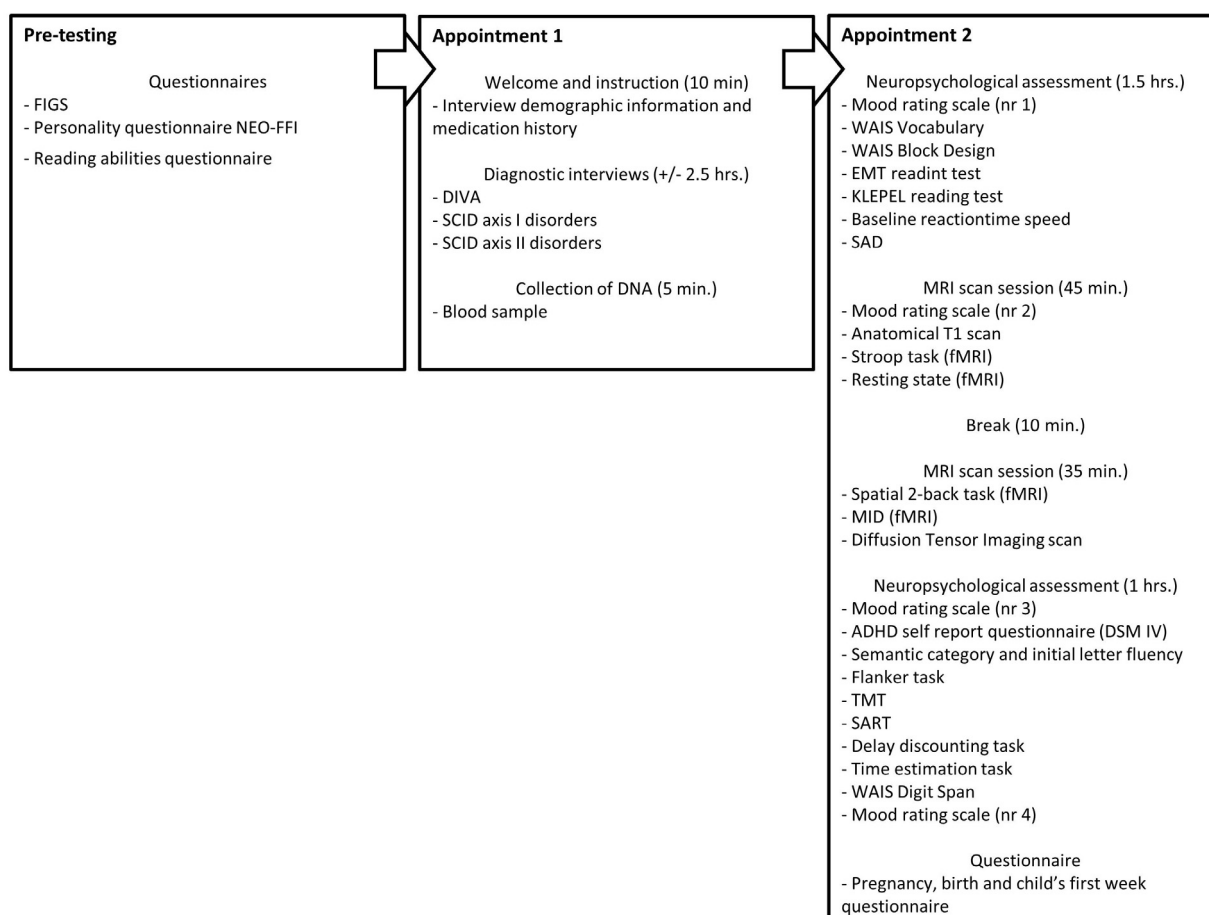
button when a 3 is presented. An omission error is made when the participant does not press the button when any digit that is not 3 is presented. A hit is made when a participant correctly responds to any digit that is not 3 (Smit et al., 2004).

Trailmaking task: In part A, participants are asked to draw a line to link numbers in consecutive order (1-2-3-...-25-26). These numbers are randomly placed on a sheet of paper. In part B, the set-shifting condition, participants are asked to draw a line to link numbers and letters in consecutive order (e.g. 1-A-2-B-3-....-K-12-L-13) (Kortte et al., 2002).

Semantic category and initial letter fluency task: In the first part, participants are asked to name as many animals as they can within one minute, afterwards they are asked to mention as many professions as they can within one minute. In the second part of the task participants are given a first-letter and are asked to mention as many words as they know that begin with that letter, again within one minute. They have 3 trials: one with the letter 'T', one with the letter 'A' and one with the letter 'D'. On these trials, it is not allowed to name any words that start with a capital letter (names, cities, countries etc.)(Hurks et al., 2004).

Delay discounting task: The participant is repeatedly asked to make a choice between two (hypothetical) incentives. One option generates an incentive (money) at a short period while the other option generates an incentive at a later time (i.e. "Do you prefer to receive 30 Euros 180 days from now, or 2 Euros immediately?"). During the task, the value of the incentives as well as the time of the delay (with which the incentive is gained) are varied. The impulsivity parameter (k) is computed from the present value of the delayed reward (V), the real value of the delayed reward (a) and the delay in days (D) with the formula: $V = a / (1+kD)$ (Dom et al., 2006).

Time estimation task: To show the length of one second, the participant is first shown a picture on a computer screen for one second, this is repeated ten times. Next, the participant has to respond to a sound (beep) by pressing the space bar one second after the sound is presented. Participants receive feedback after each trial on the accuracy of their timing ('too slow', 'too fast', 'correct')(Rommelse et al., 2008).



Supplementary Figure 1. Overview of the testing procedures of the IMpACT study.

Supplementary Table 1. List of reasons for exclusion of participants (N = 33)

Healthy controls (N = 11)	ADHD patients (N = 22)
Current axis I disorder (N = 4)	Current depression (N = 6)
Current axis II disorder (N = 2)	Use of SSRI's (N = 7)
ADHD diagnosis (DIVA symptoms) (N = 3)	Current substance use disorder (N = 3)
Other (N = 2)	IQ < 70 (N = 1)
	No ADHD (1 symptom on DIVA) (N = 1)
	Other (N = 4)

Supplementary Table 2. Psychiatric characteristics of IMpACT database (N = 265)

		Healthy controls Count (%)	ADHD patients Count (%)
Major depression ^a	Current		
	In remission	14 (10.6)	55 (41)
Dystymic disorder ^a	Current		5 (3.8)
	In remission		
Manic episode ^a	Current		
	In remission		1 (0.8)
Moment Psychotic symptoms ^a	Current		
	In remission		4 (3)
Substance abuse ^a	Current		1 (0.8)
	In remission	6 (4.5)	23 (17.3)

Anxiety disorder ^a	Current		9 (6.8)
	In remission	4 (3)	16 (12)
Obsessive compulsive disorder ^a	Current		1 (0.8)
	In remission		2 (1.5)
Post-traumatic stress syndrome ^a	Current		2 (1.5)
	In remission	4 (3)	5 (3.8)
Eating disorder ^a	Current		
	In remission	1 (0.8)	4 (3)
Other ^a	Current		2 (1.5)
	In remission		
Avoidant personality disorder ^b			2 (1.7)
Obsessive-compulsive personality disorder ^b			11 (9.1)
Passive-aggressive personality disorder ^b			1 (0.8)
Depressive personality disorder ^b			2 (1.7)
Paranoid personality disorder ^b			1 (0.8)
Schizotypal personality disorder ^b			1 (0.8)
Narcissistic personality disorder ^b			1 (0.8)
Borderline personality disorder ^b			10 (8.3)
Antisocial personality disorder ^b			6 (5)

^aAs measured by the Structured Clinical Interview for DSM-IV for axis I disorders (Groenestijn et al., 1999).

^bAs measured by the Structured Clinical Interview for DSM-IV for axis II disorders (Weertman et al., 2000).

Supplementary Table 3. Treatment characteristics of ADHD patients in the IMpACT database (N = 133)

Group	Medication class	N	Treatment duration in months Mean (SD)
Medication naïve	-	20	-
	Amphetamine stimulant	14	35 (27)
	Methylphenidate stimulant	69	27 (28)
	Nonstimulant	4	55 (76)
	Antidepressant	5	61 (60)
	Amphetamine and antidepressant	1	11
	Methylphenidate and antidepressant	2	42 (42)
	Nonstimulant and antidepressant	0	-
Past treatment	Unknown	18	13 (16)

Supplementary Table 4. Backward stepwise binary logistic regression predicting diagnosis from 17 variables

Variable in model	Backward model		
	B (SE)	Wald χ^2	p-value
Digit span - forward	-0.198 (0.088)	5.049	.025
Flanker - Total SD of RT	0.010 (0.004)	5.796	.016
SAdots - SD series errors	2.959 (0.972)	9.271	.002
SAdots - Response Bias	0.067 (0.030)	5.090	.024
Time Estimation – median absolute deviation	0.008 (0.004)	3.343	.067
Delay Discounting - k100	0.272 (0.124)	4.83	.028

Reported are the variables that significantly contributed to the model. R^2 (Nagelkerke) = 0.387, $\chi^2 = 57.542$ (df 6), $p < 0.001$.

Supplementary Table 5. Comparisons between healthy controls (HC, N = 132), ADHD patients who were medication-naïve (ADHD-, N = 20), and ADHD patients (had) used medication (ADHD+, N =83)

NPO Task	Variable	HC Mean (SD)	ADHD- ^a Mean (SD)	ADHD+ ^a Mean (SD)	ANCOVA ^b (main effect of group) F (df)	Significant <i>post-hoc</i> contrasts
1. Digit span test HC: N = 132 ADHD-: N = 19 ADHD+: N = 80	Forward score	9.83 (2.36)	9.32 (2.34)	9.07 (1.76)	3.83 (226)*	HC > ADHD+*
	Backward score	7.49 (2.34)	7.42 (2.29)	6.60 (2.07)	5.06 (226)*	HC > ADHD+*
2. Baseline speed task (ANT) HC: N = 130 ADHD-: N = 19 ADHD+: N = 81	Mean RT	313.28 (49.42)	314.11 (46.79)	311.74 (55.17)	0.004 (225)	
	SD of RT ^c	4.08 (0.53)	4.35 (0.52)	4.16 (0.48)	2.98 (225)	
3. Flanker task (ANT) HC: N = 127 ADHD-: N = 18 ADHD+: N = 76	Total mean RT	525.37 (73.00)	519.63 (69.71)	528.66 (94.38)	0.39 (216)	
	Total SD of RT	93.74 (37.04)	108.88 (43.22)	112.09 (51.45)	4.71 (216)*	HC < ADHD+*
	Inhibition RT	28.44 (28.25)	23.28 (32.31)	22.62 (38.69)	0.87 (216)	
	Inhibition errors	0.68 (1.48)	0.33 (1.50)	0.58 (1.44)	0.47 (216)	
4. SA-dots (ANT) HC: N = 128 ADHD-: N = 20 ADHD+: N = 75	Mean series completion time	899.05 (129.21)	939.95 (184.70)	933.41 (192.25)	1.55 (218)	
	SD completion time ^c	3.81 (0.44)	4.09 (0.53)	4.06 (0.52)	6.90 (218)**	HC < ADHD+**
	SD errors ^c	0.70 (0.19)	0.88 (0.26)	0.84 (0.26)	12.66 (218)***	HC < ADHD-** HC < ADHD+***
	Response bias	5.05 (6.10)	9.25 (9.87)	9.44 (10.05)	8.34 (218)***	HC < ADHD-* HC < ADHD+**
5. SART	Commission	9.31 (5.03)	8.88 (4.24)	11.10 (5.10)	2.05 (185)	

HC: N = 110	errors (N)					
ADHD-: N = 17	Omission errors (N)	2.63 (3.57)	2.53 (3.94)	4.25 (4.76)	2.91 (185)	
ADHD+: N = 63	Mean RT	315.50 (57.48)	338.07 (63.18)	311.28 (49.81)	1.07 (185)	
	hits					
	SD or RT ^c	4.35 (0.36)	4.54 (0.25)	4.51 (0.44)	4.02 (185)*	HC < ADHD+*
6. Fluency	Category;	27.76 (5.77)	25.42 (6.70)	26.15 (5.52)	2.69 (228)	
HC: N = 132	Animals					
ADHD-: N = 19	Category:	20.27 (5.23)	18.84 (5.12)	20.21 (5.29)	0.83 (228)	
	professions					
ADHD+: N = 82	Letters	41.91 (10.51)	38.79 (12.65)	38.91 (10.51)	2.47 (228)	
7. Time estimation	Median response	1004.73 (72.41)	959.94 (49.53)	1008.59 (80.34)	3.19 (211)*	HC > ADHD-* ADHD+ > ADHD-*
HC: N = 127	time					
ADHD-: N = 18	Median response	51.30 (51.12)	53.17 (34.04)	62.90 (50.17)	1.26 (211)	
ADHD+: N = 71	time					
	absolute deviation from 1000ms					
8. Delay Discounting	K 100 ^c	-5.25 (1.54)	-4.32 (2.03)	-4.55 (1.60)	5.57 (200)**	HC < ADHD+*
	K 30 ^c	-4.76 (1.65)	-4.15 (1.61)	-4.47 (1.75)	1.40 (200)	
HC: N = 123	K 10 ^c	-4.39 (1.43)	-3.89 (2.13)	-3.91 (1.60)	2.49 (200)	
ADHD-: N = 16						
ADHD+: N = 66						
9.	Part A	23.70 (7.51)	28.42 (8.73)	25.81 (7.99)	4.66 (226)*	HC < ADHD-*
Trailmaking task	Part B	50.06 (17.30)	52.95 (15.36)	56.32 (17.99)	4.28 (224)*	HC < ADHD-*
HC: N = 132	Part B - A	26.33 (13.51)	24.53 (14.47)	30.38 (15.85)	3.02 (224)	
ADHD-: N = 19						
ADHD+: N = 80						

^a ADHD- = medication-naïve patient with ADHD; ADHD+ = non medication-naïve patient with ADHD.

^b covariates age and gender.

^c log-transformed variable to normal distribution.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.002$

Supplementary Table 6. Comparisons between healthy controls (HC), ADHD patients without prior episodes of MDD (ADHD-ND), and ADHD with past MDD (ADHD-D)

NPO Task	Variable	HC Mean (SD)	ADHD-ND ^a Mean (SD)	ADHD-D ^a Mean (SD)	ANCOVA ^b (main effect of group) F (df)	Significant <i>post-hoc</i> contrasts
1. Digit span test	Forward score	9.77 (2.42)	9.12 (2.04)	8.92 (1.96)	3.53 (2, 225)*	
HC: N = 112	Backward score	7.40 (2.27)	6.50 (2.25)	6.75 (2.27)	3.98 (2, 225)*	HC > ADHD-ND *
ADHD-ND: N = 66						
ADHD -D: N = 52						
3. Baseline	Mean RT	313.54	313.00	316.94	0.06 (2, 225)	

speed task (ANT) HC: N = 111 ADHD-ND: N =66 ADHD -D: N = 53	SD of RT ^c	(48.79) 4.06 (0.52)	(52.26) 4.32 (0.53)	(60.62) 4.20 (0.60)	6.31 (2,225)**	HC < ADHD-ND **
4. Flanker task (ANT) HC: N = 107 ADHD-ND: N =63 ADHD -D: N = 50	Total mean RT Total SD of RT Inhibition RT Inhibition errors	524.14 (67.78) 91.44 (33.21) 26.77 (25.43) 0.78 (1.51)	537.67 (83.18) 125.01 (66.96) 23.94 (47.29) 0.49 (1.60)	530.64 (104.07) 109.80 (48.20) 21.38 (32.04) 0.84 (1.38)	0.853 (2,215) 9.64 (2,215)*** 0.45 (2,215) 0.80 (2,215)	HC < ADHD-ND ***
5. SA-dots (ANT) HC: N = 108 ADHD-ND: N =63 ADHD -D: N = 51	Mean series completi on time SD completi on time ^c SD errors ^c	898.61 (119.95) 3.81 (0.43) 0.70 (0.19)	926.22 (176.10) 4.02 (0.55) 0.85 (0.27)	948.57 (154.92) 4.11 (0.46) 0.90 (0.23)	1.88 (2,217) 8.56(2,217)* ** 17.86 (2,217)***	HC < ADHD-ND * HC < ADHD-D *** HC < ADHD-ND *** HC < ADHD-D *** HC < ADHD-ND ** HC < ADHD-D*
6. SART HC: N = 98 ADHD-ND: N =54 ADHD -D: N = 41	Response bias Commissio n errors (N) Omission errors (N) Mean RT hits (Go- trials) SD or RT on Go trials ^c	5.68 (6.23) 9.46 (5.06) 2.59 (3.49) 313.32 (53.41) 4.34 (0.36)	9.67 (10.13) 11.20 (4.97) 4.39 (4.72) 320.24 (60.25) 4.59 (0.43)	9.61 (9.51) 9.41 (4.63) 3.39 (4.59) 331.75 (58.53) 4.48 (0.45)	7.09 (2,217)*** 2.45 (2,188) 3.31 (2,188)* 1.81 (2,188) 6.90 (2,188)***	HC < ADHD-ND ** HC < ADHD-D* HC < ADHD-ND * HC < ADHD-ND **
7. Fluency HC: N = 112 ADHD-ND: N =67 ADHD -D: N = 54	Category; Animals Category: profession s Letters	27.85 (5.87) 20.63 (5.59) 42.15 (10.92)	25.76 (5.88) 20.12 (5.77) 38.03 (11.35)	25.98 (6.27) 19.52 (4.54) 40.37 (10.53)	3.20 (2,228)* 0.78 (2,228) 3.09 (2,228)*	HC > ADHD-ND *
8. Time estimation HC: N = 107 ADHD-ND: N =60 ADHD -D: N = 45	Median response time Median response time absolute deviation from 1000ms	1004.88 (69.13) 49.58 (48.18)	995.90 (85.35) 67.57 (51.56)	996.91 (81.95) 61.09 (53.93)	0.44 (2,207) 2.88 (2,207)	

9. Delay Discounting	K 100	-5.30 (1.46)	-4.51 (1.72)	-4.42 (1.52)	7.37 (2,201)***	HC < ADHD-ND **
HC: N = 106						HC < ADHD-D **
ADHD-ND: N = 56	K 30	-4.71 (1.53)	-4.34 (1.79)	-4.46 (1.52)	1.13 (2,201)	
ADHD -D: N = 44	K 10	-4.34 (1.30)	-3.89 (1.86)	-3.94 (1.36)	2.21(2,201)	
10. Trailmaking task	Part A	23.15 (7.11)	27.38 (8.79)	26.43 (8.00)	7.83 (2,225)***	HC < ADHD-ND **
HC: N = 112	Part B	49.54 (17.06)	56.52 (19.21)	61.00 (22.44)	7.44 (2,224)***	HC < ADHD-D *
ADHD-ND: N = 65	Part B - A	26.39 (13.50)	29.14 (17.43)	34.37 (20.06)	4.18 (2,224)*	HC < ADHD-ND*
ADHD -D: N = 53						HC < ADHD-D **

^a MDD = major depressive disorder. ADHD with MDD refers to patients that have MDD in remission (i.e. no current symptoms of depression).

^b Covariates age and gender.

^c Log-transformed variable to normal distribution.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.002$

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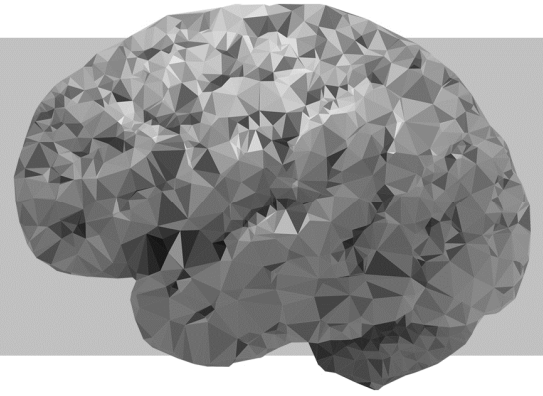
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Brain alterations in adult ADHD: Effects of gender, treatment and comorbid depression

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Abstract

Children with attention-deficit/hyperactivity disorder (ADHD) have smaller volumes of total brain matter and subcortical regions, but it is unclear whether these represent delayed maturation or persist into adulthood. We performed a structural MRI study in 119 adult ADHD patients and 107 controls and investigated total gray and white matter and volumes of accumbens, caudate, globus pallidus, putamen, thalamus, amygdala and hippocampus. Additionally, we investigated effects of gender, stimulant treatment and history of major depression (MDD). There was no main effect of ADHD on the volumetric measures, nor was any effect observed in a secondary voxel-based morphometry (VBM) analysis of the entire brain. However, in the volumetric analysis a significant gender by diagnosis interaction was found for caudate volume. Male patients showed reduced right caudate volume compared to male controls, and caudate volume correlated with hyperactive/impulsive symptoms. Furthermore, patients using stimulant treatment had a smaller right hippocampus volume compared to medication-naïve patients and controls. ADHD patients with previous MDD showed smaller hippocampus volume compared to ADHD patients with no MDD. While these data were obtained in a cross-sectional sample and need to be replicated in a longitudinal study, the findings suggest that developmental brain differences in ADHD largely normalize in adulthood. Reduced caudate volume in male patients may point to distinct neurobiological deficits underlying ADHD in the two genders. Smaller hippocampus volume in ADHD patients with previous MDD is consistent with neurobiological alterations observed in MDD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in childhood and is strongly persistent over time (Polanczyk et al., 2007). At least 15% of affected children still meets full ADHD criteria according to the DSM-IV in adulthood, and 40-60% remits only partially (Faraone et al., 2006). The average prevalence of adult ADHD is estimated to be between 2.5% and 4.9% (Simon et al., 2009).

Magnetic Resonance Imaging (MRI) studies have reported structural abnormalities in several regions of the brain in patients with childhood ADHD compared to controls (see meta-analyses by (Ellison-Wright et al., 2008; Frodl and Skokauskas, 2012; Nakao et al., 2011; Valera et al., 2007). However, there are inconsistencies across studies and it is unclear which brain regions have the strongest reduction of volumes or area compared to controls. An often used strategy to overcome limitations of single studies is to perform a meta-analysis. So far, four meta-analyses of structural MRI studies have been published. The first restricted the analysis to childhood studies and pooled data of 22 region of interest studies comparing 565 ADHD children and 583 controls (Valera et al., 2007). The largest reductions in ADHD compared to controls were found in the cerebellar regions, the splenium of the corpus callosum, total and right cerebral volumes and right caudate nucleus. The second meta-analysis also focused on childhood studies and included seven whole-brain voxel-based morphometry (VBM) studies comparing 114 children with ADHD and 143 comparison subjects; the authors reported that ADHD was associated with gray matter reductions in right putamen and globus pallidus (Ellison-Wright et al., 2008).

An important question is whether brain abnormalities observed in childhood ADHD persist into adulthood. In trying to answer this question, two meta-analyses included pediatric and adult ADHD samples to examine age effects on gray matter volume using VBM. Nakao et al. (2011) included 202 children and adolescents, 176 adults with ADHD and 344 healthy controls, while Frodl and Skokauskas (2012) examined 175 children and adolescents and 145 adult patients with ADHD, plus 288 healthy controls. Both studies confirmed findings from previous meta-analyses that volume reductions of the right globus pallidus and putamen volumes as well as the right and left volumes of the caudate were the most consistent abnormalities in childhood ADHD. Using meta-regression analysis, Nakao and colleagues further showed that differences in gray matter volume in the right putamen disappeared with increasing age suggesting normalization in adulthood. An earlier cross-sectional study in 152 children and adolescents with ADHD and 139 age- and sex-matched controls had also suggested normalization of caudate volume throughout adolescence (Castellanos et al., 2002). Along the same lines, a longitudinal study investigating cortical thickness in 223 ADHD children and 223 normally developing children showed that the peak of cortical thickness maturation was delayed in children with ADHD compared with healthy peers by an average of 3 years, with some regions, including frontal and temporal cortex areas, being delayed in their maturation by up to 5 years (Shaw et al., 2007). A recent longitudinal study in 234 children with ADHD and 231 typically developing children by the same group extended the previous finding by demonstrating that maturation of cortical

surface area was delayed in the same way as cortical thickness was (Shaw et al., 2012). However, neither of the longitudinal studies was able to examine whether persistence of ADHD is related with brain maturation, since most of their participants were still under 18 years and adult clinical outcome data were lacking for the majority of subjects. Normalization may still only concern the children/adolescents with ADHD who show recovery in adulthood, because several structural neuroimaging studies in adult ADHD patients did observe differences compared to controls (Table 1). However, sample sizes have been relatively small. So far, 13 studies have been published with sample sizes ranging from 8 (Hesslinger et al., 2002) to 74 ADHD patients (Seidman et al., 2011). Three studies in adult ADHD patients show reductions in the caudate nucleus (Almeida Montes et al., 2010; Proal et al., 2011; Seidman et al., 2011). Additional volumetric reductions are found in putamen (Seidman et al., 2011), right thalamus (Proal et al., 2011), dorso-lateral prefrontal cortex (Biederman et al., 2008; Makris et al., 2007; Seidman et al., 2011; Seidman et al., 2006), left fronto-orbital cortex (Hesslinger et al., 2002) and anterior cingulate cortex (Amico et al., 2011; Biederman et al., 2008; Makris et al., 2007), superior and inferior frontal gyrus (Almeida et al., 2010; Depue et al., 2010; Seidman et al., 2006), early visual cortex (Ahrendts et al., 2011), amygdala (Frodal et al., 2010) and cerebellar regions (Biederman et al., 2008; Proal et al., 2011; Seidman et al., 2011).

Table 1. Summary of study characteristics and results

Study	Subjects	Gender (% male)	Age ADHD: range of M (SD)	Analysis method	Findings
Hesslinger et al., 2002	8 ADHD, 17 HC	100	31.4 (4.4)	Manual defined ROI: orbital frontal cortex	ADHD < HC, left orbital frontal cortex
Seidman et al., 2006	24 ADHD, 18 HC	50	38.0 (2.2)	Manual parcellation of the neocortex	ADHD < HC, dorsolateral, prefrontal and anterior cingulate cortex
Makris et al., 2007	24 ADHD, 18 HC	50	38 (2.2)	Cortical thickness	ADHD < HC, right hemisphere: inferior parietal lobule, the dorsolateral prefrontal, and the anterior cingulate cortices
Biederman et al., 2008	26 ADHD, 23 HC, 18 BPD, 31 ADHD + BPD	50	36.9 (11.1)	Manual parcellation of the neocortex	ADHD < HC, neocortex, overall frontal lobe and superior prefrontal cortex, right anterior cingulate cortex and cerebellum
Perlov et al., 2008	27 adult, 27 HC	63	32.4 (10.6)	Manual defined ROI: hippocampus and amygdala	ADHD = HC
Almeida et al., 2010	21 ADHD children, 18 ADHD adolescents, 20 ADHD adults,	50	28.95 (4.01)	Cortical thickness	Children, adolescents & adult ADHD < HC, regions in the right superior frontal gyrus

	22 HC children, 20 HC adolescents, 20 HC adults					
Depue et al., 2010	30 ADHD, 20 HC	61	20.0 (1.7)	VBM. Whole brain, ROI: the superior parietal lobule, right inferior frontal gyrus	ADHD = HC, whole brain. ADHD < HC, right inferior frontal gyrus	
Frodal et al., 2010	20 ADHD, 20 HC, 20 MD	75	33.6 (10.2)	Manual defined ROI: hippocampus and amygdala	ADHD < HC & MD, bilateral amygdala	
Montes et al., 2010	20 ADHD, 20 HC	50	28.95 (4.01)	VBM	ADHD < HC, caudate	
Ahrendts et al., 2011	31 ADHD, 31 HC	65	31.20 (9.70)	VBM	ADHD < HC, visual cortex	
Amico et al., 2011	20 ADHD, 20 HC	75	33.6 (10.2)	VBM. ROI: prefrontal cortex, cingulate cortex, hippocampus and amygdala	ADHD = HC, whole brain. ADHD < HC, anterior cingulate cortex	
Proal et al., 2011	59 childhood ADHD, 80 HC. 17 persistent ADHD , 26 remitted ADHD	100	41.1 (2.7)	VBM and cortical thickness	ADHD < HC, total cerebral volume, global cortical thickness, right caudate, right thalamus, and bilateral cerebellar hemispheres	
Seidman et al., 2011	74 ADHD, 54 HC	51	37.3 (12.6)	VBM. Whole brain, ROI: dorsolateral prefrontal cortex, anterior cingulate cortex, caudate, putamen, inferior parietal lobule, and cerebellum	ADHD = HC, whole brain. ADHD < HC, dorsolateral prefrontal cortex, anterior cingulate cortex, caudate, putamen, inferior parietal lobule, and cerebellum	

Taken together, the literature does not provide a clear picture how abnormal childhood brain volumetrics translate into adulthood. Moreover, it remains unclear what the role of gender is in this developmental process. In childhood ADHD, relatively more boys than girls are diagnosed with ADHD, with male-to-female ratios ranging from 3:1 (Arnold, 1996) to as much as 9:1 (Gaub and Carlson, 1997). As an unfortunate consequence, affected girls have been extremely underrepresented in childhood neuroimaging studies (Valera et al., 2007), and the studies that specifically examined gender differences showed inconsistent findings. Castellanos et al. (2002) showed that boys (n=89) and girls (n=63) have relatively similar structural brain abnormalities. In contrast, Qiu et al. (2009) examined the effects of ADHD, gender and their interaction on basal ganglia volume in 27 boys and 20 girls with

ADHD and reported no abnormalities in girls, while boys showed smaller volumes of caudate, putamen and globus pallidus. In the meta-analysis of Frodl and Skokauskas (2012), smaller right putamen volumes in childhood ADHD did not reach significance when gender was a covariate. This indicates that in childhood ADHD gender may indeed play a role. Compared to childhood, the prevalence of ADHD in adults shows a more balanced gender distribution (Biederman et al., 1994; Biederman et al., 1993). Most structural neuroimaging studies of adult ADHD included comparable numbers of males and females. Some reported absence of gender differences (Almeida Montes et al., 2010; Seidman et al., 2011), but most studies may have lacked power to adequately examine such differences because of their limited sample size.

A confounding factor to nearly all ADHD studies is medication use. Stimulants like methylphenidate are currently the most frequently prescribed medications for ADHD (Heal et al., 2009), and are known to alter brain activity (Epstein et al., 2007). Studies examining the effects of stimulants on structural brain measures indicate that psychostimulants may not have a severe effect on the brain. In children with ADHD, no significant differences in brain volumes were found between medicated and treatment-naïve children (Castellanos et al., 2002). Other studies reported that chronic stimulant treatment is associated with normal volumes of brain structures, such as right anterior cingulate and cerebellar vermis (Bledsoe et al., 2009; Pliszka et al., 2006). Moreover, ADHD adolescents not receiving treatment showed more rapid cortical thinning compared with patients taking psychostimulants (Shaw et al., 2009). Two recent meta-regression analyses reported that stimulant use is associated with an absence of reductions of regional gray matter volumes using VBM, suggesting a normalizing effect of treatment on neural abnormalities (Frodl and Skokauskas, 2012; Nakao et al., 2011).

Neuroimaging research addressing the effects of comorbidity between ADHD and major depressive disorder (MDD) is scarce, even though MDD co-occurs with adult ADHD in up to 50% of cases (McIntosh et al., 2009). There is strong evidence that MDD is related with structural brain changes in the hippocampus and amygdala. Several meta-analyses demonstrated a reduction in hippocampal volume in patients relative to controls (Arnone et al., 2012; Cole et al., 2011; Du et al., 2012; Kempton et al., 2011; Koolschijn et al., 2009; Videbech and Ravnkilde, 2004). For amygdala volume, a meta-analysis showed that only unmedicated, depressed individuals have decreased amygdala volume relative to healthy controls (Hamilton et al., 2008). However, there is also evidence that amygdala volume may be enlarged in acute first episode MDD (van Eijndhoven et al., 2009). So far, three structural imaging studies examined current depression severity in adult ADHD. Perlov et al. (2008) investigated depression severity in a group of 27 patients and found a correlation between enlarged amygdala volume and depressive symptoms. Frodl et al. (2010) examined amygdala and hippocampal volumes in 20 patients with ADHD, 20 matched patients with MDD and 20 healthy controls. Hippocampal volumes were unaltered in ADHD patients and although patients had smaller amygdala volumes compared with the other groups, they demonstrated that higher rates of depressive symptoms in ADHD patients were related with

larger amygdala volumes. Amico et al. (2011) studied depression severity in a sample of 20 ADHD adults, but found no correlation with structural alterations. In sum, the limited literature investigating depressive symptoms in ADHD does not support evidence for smaller hippocampal volume, and two studies suggest that larger amygdala volume is associated with higher current depression scores (Frodl et al., 2010; Perlov et al., 2008).

To shed more light on the issues discussed above, we investigated a large sample of 119 adult ADHD patients and 107 healthy comparison subjects. We were particularly interested in subcortical gray matter structures, which have been consistently found altered in persistent ADHD. This is in line with the hypothesis of Halperin and Schulz (2006), which provides a neurodevelopmental model for ADHD persistence proposing that the disorder is caused by non-cortical neural dysfunction that is present early in life and remains relatively stable throughout the lifetime, potentially compensated by prefrontal cortex function in remitting forms of ADHD. We used the automated FSL FIRST subcortical segmentation tool to compare regional volumetric data for a range of deep gray matter structures. A reduced caudate nucleus volume is one of the most consistent findings in childhood literature (Frodl and Skokauskas, 2012; Nakao et al., 2011; Valera et al., 2007), and while some studies suggest that this volume difference may disappear during adolescence (Ahrendts et al., 2011; Amico et al., 2011; Carmona et al., 2005; Castellanos et al., 2002; Depue et al., 2010), several studies still find reduced caudate volume in adult ADHD patients (Almeida Montes et al., 2010; Proal et al., 2011; Seidman et al., 2011). Moreover, caudate volume was found to be significantly smaller in patients in the largest MRI study performed in adult ADHD to this date (N=74). The results from Proal et al. (2011) nicely support the hypothesis by Halperin and Schulz by showing that caudate volume differences were present independent of whether ADHD persisted or remitted. Based on this evidence, we hypothesized that caudate volume differences are still present in adults with ADHD. In addition, we explored other brain structures for differences between patients and controls and the role of gender herein, expecting structural differences to be less pronounced in females than in males (Frodl and Skokauskas, 2012; Qiu et al., 2009). For differences observed, we explored their clinical relevance for symptom severity in ADHD. To test for additional regions of altered gray matter volume between patients and controls not captured by FSL FIRST, we also performed a voxel-based morphometry (VBM) analysis of the entire brain. We subsequently examined stimulant treatment effects on the subcortical brain volumes expecting to find that treatment-naïve patients show smaller brain volumes as compared to patients taking medication (Frodl and Skokauskas, 2012; Nakao et al., 2011). Lastly, effects of a history of MDD in ADHD were investigated, since our sample contained enough ADHD patients diagnosed with previous MDD to reach an adequate sample size. Here, we focused on hippocampal and amygdala volumes for their known importance in depression. We expected smaller hippocampal volume in patients with a history of MDD (Arnone et al., 2012; Cole et al., 2011; Du et al., 2012; Kempton et al., 2011; Koolschijn et al., 2009; Videbech and Ravnkilde, 2004). Since we collected no information on current depression, we tested amygdala volume differences in an exploratory fashion.

Experimental procedures

Participants

228 individuals (119 adult ADHD patients, 107 healthy comparison subjects) from the Dutch cohort of the International Multicentre persistent ADHD CollaboraTion, IMpACT (Franke et al., 2010a), participated in this study. The ADHD patients and the age-, gender-, and IQ-comparable group of healthy comparison subjects were recruited from the department of Psychiatry of the Radboud University Nijmegen Medical Centre and through advertisements. For the analyses of stimulant treatment effects, we split the ADHD group into a group of medication naive patients and a group of patients receiving stimulant treatment. Eight ADHD subjects receiving atomoxetine and 13 patients who had previously received medication and had stopped medication well before testing were excluded from this part of the analysis.

Patients were included if they met DSM-IV-TR criteria for ADHD in childhood as well as adulthood. All participants were assessed using the Diagnostic Interview for Adult ADHD (DIVA) (Kooij, 2010). This interview focuses on the 18 DSM-IV symptoms of ADHD and uses concrete and realistic examples to thoroughly investigate whether a symptom is currently present or was present in childhood. In order to obtain information about ADHD symptoms and impairment in childhood, additional information was acquired from parent and school reports, whenever possible. The Structured Clinical Interview for DSM-IV (SCID-I & SCID-II) (Groenestijn et al., 1999; Weertman et al., 2000) was used for comorbidity assessment. Assessments were carried out by trained professionals (psychiatrists or psychologists). In addition, a quantitative measure of clinical symptoms was obtained using the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

Exclusion criteria for participants were psychosis, alcohol or substance use disorder in the last 6 months, current major depression, full-scale IQ estimate <70 (prorated from Block Design and Vocabulary of the Wechsler Adult Intelligence Scale-III), neurological disorders, sensorimotor disabilities, non-Caucasian ethnicity, and medication use other than psychostimulants or atomoxetine. An additional exclusion criterion for the healthy comparison subjects was a current neurological or psychiatric disorder according to SCID-I. This study was approved by the regional ethics committee. Written informed consent was obtained from all participants.

MRI acquisition and data processing

T1-weighted images were acquired using a 1.5T MRI scanner (Sonata Siemens, Munich, Germany) at the Donders Centre for Cognitive Neuroscience. All scans covered the entire brain and had a voxel size of $1 \times 1 \times 1 \text{ mm}^3$, TR 2730 ms, TI 1000 ms, TE 2.95 ms, 176 sagittal slices, field of view 256 mm.

For automatic segmentation of subcortical brain structures, the FIRST module (version 1.2) of FSL (version 4.1) was used (www.fmrib.ox.ac.uk/fsl/first/index.html). This method is based on Bayesian statistical models of shape and appearance for seventeen subcortical structures from 317 manually labelled T1-weighted MR images. To fit the models, the probability of the shape given the observed intensities was used (Smith et al., 2004). In

addition, to model intensity at the structural boundary, automatic boundary correction was applied. The scan–rescan reliability of FSL derived volumes is about 0.9 or higher for large structures such as the thalamus and caudate, but is smaller (0.6) for smaller structures such as the accumbens, pallidum and amygdala (Morey et al., 2010; Narayana et al., 1988). After automatic segmentation, volume determination of the subcortical structures was calculated using a script in Matlab7.2 (MathWorks, USA). In this script the volumes of the regional structures of interest were calculated by multiplying the number of voxels with the voxel volume (1 mm^3). Inspection of the segmented subcortical structures projected onto the T1-weighted MRI scans was performed using the software MRlcroN Version Beta 7 (www.mricro.com/mricron) to detect obvious segmentation errors.

Whole brain segmentation of gray matter, white matter and cerebrospinal fluid (CSF) was performed using the VBM8 toolbox in SPM8. Total volume of gray and white matter was calculated by adding the resulting tissue probability maps. Total brain volume was defined as the sum of white and gray matter volume.

A post-hoc VBM analysis was performed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>) and Matlab7.2 (MathWorks, USA). First, MR images were segmented into gray matter, white matter and CSF using the standard unified segmentation model in SPM8 (Ashburner and Friston, 2005). Second, gray matter population templates were generated from the entire image dataset using the diffeomorphic anatomical registration using exponentiated Lie algebra (DARTEL) technique (Ashburner, 2007). After an initial affine registration of the gray matter DARTEL templates to the tissue probability maps in Montreal Neurological Institute (MNI) space (<http://www.mni.mcgill.ca/>), non-linear warping of gray matter images was then performed to the DARTEL gray matter template in MNI space. Subsequently images were modulated to ensure that relative volumes of gray matter were preserved following the spatial normalisation procedure. Lastly, images were smoothed with an 8 mm full width at half maximum Gaussian kernel. After spatial pre-processing, the smoothed, modulated, normalised gray matter datasets were used for statistical analysis (absolute threshold: 0.2).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20 (SPSS, Inc., Chicago, IL). Differences for total gray and white matter were tested with separate general linear models (GLM) with diagnosis and gender as fixed factors. Age and total white matter volume (when correcting for gray matter) and total gray matter volume (when correcting for white matter) were included in the model as co-variables. To compare volumetric data of our regions of interests (left and right nucleus accumbens, amygdala, caudate nucleus, hippocampus, globus pallidus, putamen and thalamus), a GLM was used in which the volumes were included as dependent factors. Diagnosis (healthy comparison subjects vs. ADHD patients), treatment (healthy comparison subjects vs. ADHD naive vs. ADHD stimulant-medicated) and depression history (ADHD patients with no history of depressive episodes vs. ADHD patients with one or more episodes in the past) were added as

between subject factors. For each between-subject factor, a separate GLM analysis was used. For all the GLM analyses, age and total brain volume were included in the model as covariates and gender was added as fixed factor to investigate interaction effects. Whenever this interaction term was significant at $\alpha = 0.05$, we analyzed the results separately by gender. The relation between total number of self-reported ADHD symptoms and right caudate volume in male patients was studied using linear regression analysis adjusting for age and total brain volume. To explore effects of the distinct ADHD symptom domains, a similar analysis was performed including either inattentive symptom count or hyperactivity/impulsivity symptom count as an independent variable. Significant between subject findings were explored using linear regression analysis to investigate the relation between volume and duration of medication use (in months) adjusting for age and total brain volume. Because data for the rating scale scores and duration of medication use were highly positively skewed, prior to the regression analyses, a logarithmic transformation was conducted because data for the rating scale scores and duration of medication use were highly positively skewed. All statistical tests were two-sided, unless stated otherwise. As we hypothesized differences in caudate volume to exist, we performed a one-sided test and did not correct for multiple testing for this structure. All other tests were corrected for multiple testing. This correction consisted of adjusting p -values based on the false discovery rate (FDR) controlling procedure with a p -value of less than 0.05 (Benjamini and Hochberg, 1995).

For the post-hoc VBM analysis, group differences in absolute grey matter volume were assessed using a full-factorial ANCOVA with diagnosis and gender included as factors and participants' age and total brain volume added to the model as covariates. The FDR correction for multiple comparisons was used with a p -value of less than 0.05. The extent threshold was set at 10 voxels, to eliminate very small clusters.

Results

A total of 119 adult ADHD patients and 107 healthy comparison subjects were included in the analysis. The demographics for this sample are summarized in Table 2. There were no significant differences in age and estimated IQ between patients and controls ($p > .05$), and sex ratio did not significantly differ between the two groups ($\chi^2 = 0.25$, $p = 0.68$). Group-by-sex ANOVAs showed group effects for number of self-reported symptoms for inattention ($F(1, 222) = 673.33$, $p > .001$) and hyperactivity/impulsivity symptoms ($F(1, 222) = 354.17$, $p > .001$) and sex effect for number of symptoms for inattention ($F(1, 222) = 9.94$, $p = .002$) but no interactions. The subjects with ADHD had higher number of self-reported symptom of inattention and hyperactivity/impulsivity and the women had higher number of self-reported symptoms for inattention. There were no significant differences in sex ratio and age and estimated IQ was not different between healthy comparison subjects, ADHD naive and ADHD stimulant-medicated patients ($p > .05$). The groups differed on symptoms for inattention and hyperactivity/impulsivity symptoms ($F(2, 199) = 344.26$, $p > .001$; $F(2, 199) = 192.51$, $p > .001$). On both scales, healthy comparison subjects, showed lower number of symptoms than ADHD naive and ADHD stimulant-medicated. There were no significant

differences in age, estimated IQ between and number of symptoms for inattention and hyperactivity/impulsivity symptoms between ADHD patients with one or more episode in the past and no history of depressive episodes ($p > .05$). The group ADHD patients with one or more episode in the past contained more females than the group ADHD patient with no history of depressive episodes ($\chi^2 = 3.94, p = .047$).

Table 2. Demographic characteristics of ADHD patients and healthy comparison subjects

Variable	Total group		Males		Females	
	HC (N = 107)	ADHD (N = 119)	HC (N = 45)	ADHD (N = 46)	HC (N = 62)	ADHD (N = 73)
Age	36.92 ± 11.54	36.29 ± 10.90	36.02 ± 11.06	37.72 ± 10.47	37.56 ± 11.92	35.40 ± 11.14
Estimated IQ ^a	110.21 ± 15.35	107.49 ± 14.69	112.11 ± 14.68	106.57 ± 14.11	108.84 ± 15.79	108.07 ± 15.11
Inattentive symptoms ^b	0.62 ± 1.12	6.43 ± 2.06	1.02 ± 1.39	6.87 ± 2.06	0.32 ± 0.76	6.15 ± 2.03
Hyperactive/Impulsive symptoms ^b	0.82 ± 1.29	5.60 ± 2.24	1.11 ± 1.56	5.63 ± 2.09	0.61 ± 1.01	5.58 ± 2.35
	N	N	N	N	N	N
One or more depressive episode (remitted) ^c	11 (10%)	55 (46%)	6 (13%)	17 (37%)	5 (9%)	38 (52%)
Bipolar (remitted) ^c	0	8 (7%)	0	3 (7%)	0	5 (7%)
Anxiety disorder (remitted) ^c	7	27 (23%)	4 (9%)	7 (15%)	3 (5%)	20 (27%)
Substance abuse (remitted) ^c	8	22 (18%)	7 (16%)	17 (36%)	1 (2%)	5 (7%)
Borderline ^c	0	10 (8%)	0	2 (4%)	0	8 (11%)
Antisocial ^c	0	3 (3%)	0	2 (4%)	0	1 (1%)
Medication-naïve	-	16 (13%)	-	9 (20%)	-	7 (10%)
On stimulant medication	-	82 (69%)	-	29 (61%)	-	53 (74%)
Medication in the past	-	13 (11%)	-	6 (13%)	-	7 (10%)
On atomoxetine	-	8 (7%)	-	2 (4%)	-	6 (8%)

Demographic information representing means ± standard deviations or percentage per group. HC = Healthy comparison subject.

^a Prorated from Block Design and Vocabulary of WAIS-III-R.

^b As measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^c As measured by the Structured Clinical Interview for DSM-IV for axis I (Groenestijn et al., 1999) and axis II (Weertman et al., 2000) disorders.

Comparison of the volumes of caudate nucleus between patients and controls showed no main effect of diagnosis on volume (Table 3). However, when probing caudate volumes for interaction effects of ADHD diagnosis and gender, right caudate volume showed a significant interaction effect ($F(1,220) = 5.84, p = .016$, partial eta squared = .026). Separate post-hoc analyses for both genders indicated that right caudate volume was significantly smaller in male ADHD patients ($F(1, 87) = 7.14, p_{\text{one-sided}} = .005$, partial eta squared = .076), while this was not the case in females ($F(1, 131) = 0.55, p_{\text{one-sided}} = .23$, partial eta squared = 0.004). Results for the analysis of the volumes of nucleus accumbens, amygdala, hippocampus, globus pallidus, thalamus, and total white and gray matter are summarized

in Table 3. No differences between ADHD patients and healthy subjects were observed for total brain volume, gray matter or white matter volume. In addition, none of the other subcortical brain volumes nor hippocampus volume differed significantly. Taking gender into account did not change these results. A post-hoc, whole-brain, voxel-by-voxel examination comparing the subjects and controls did not reveal any areas with significant gray matter differences, nor was there no significant effect of diagnosis x gender interaction ($p < 0.05$, FDR corrected).

Table 3. Bilateral and total volumes (in ml) \pm standard error for ADHD patients (ADHD) and healthy comparison subjects (HC) based on the estimated marginal means and controlled for age, gender and total brain volume. Volumes are also shown for males (M) and females (F), separately

	HC (N = 107)	HC M (N = 45)	HC F (N = 62)	ADHD (N = 119)	ADHD M (N = 46)	ADHD F (N = 73)
L CDN	2.60 \pm 0.03	2.74 \pm 0.04	2.51 \pm 0.03	2.57 \pm 0.02	2.65 \pm 0.04	2.51 \pm 0.03
R CDN	2.69 \pm 0.03	2.88 \pm 0.04	2.56 \pm 0.03	2.65 \pm 0.02	2.74 \pm 0.04	2.59 \pm 0.03
L ACC	0.34 \pm 0.01	0.37 \pm 0.01	0.33 \pm 0.01	0.34 \pm 0.01	0.37 \pm 0.01	0.33 \pm 0.01
R ACC	0.37 \pm 0.01	0.40 \pm 0.01	0.36 \pm 0.01	0.36 \pm 0.01	0.38 \pm 0.01	0.35 \pm 0.01
L AMY	1.11 \pm 0.01	1.19 \pm 0.02	1.06 \pm 0.02	1.10 \pm 0.01	1.20 \pm 0.02	1.04 \pm 0.02
R AMY	1.09 \pm 0.01	1.18 \pm 0.02	1.02 \pm 0.02	1.10 \pm 0.01	1.21 \pm 0.02	1.04 \pm 0.02
L HIP	2.76 \pm 0.03	2.97 \pm 0.04	2.62 \pm 0.03	2.77 \pm 0.02	2.92 \pm 0.04	2.66 \pm 0.03
R HIP	2.69 \pm 0.02	2.85 \pm 0.04	2.57 \pm 0.03	2.66 \pm 0.02	2.80 \pm 0.04	2.57 \pm 0.03
R GP	0.92 \pm 0.01	1.00 \pm 0.01	0.86 \pm 0.01	0.92 \pm 0.01	0.99 \pm 0.01	0.88 \pm 0.01
L GP	0.96 \pm 0.01	1.02 \pm 0.01	0.91 \pm 0.01	0.96 \pm 0.01	1.03 \pm 0.01	0.91 \pm 0.01
L PUT	3.01 \pm 0.03	3.25 \pm 0.04	2.84 \pm 0.04	3.02 \pm 0.03	3.22 \pm 0.04	2.88 \pm 0.03
R PUT	3.06 \pm 0.03	3.31 \pm 0.05	2.88 \pm 0.04	3.01 \pm 0.03	3.21 \pm 0.04	2.89 \pm 0.04
L THA	5.43 \pm 0.03	5.70 \pm 0.05	5.25 \pm 0.04	5.43 \pm 0.03	5.70 \pm 0.05	5.25 \pm 0.04
R THA	5.77 \pm 0.03	6.06 \pm 0.05	5.56 \pm 0.04	5.71 \pm 0.03	5.97 \pm 0.05	5.54 \pm 0.07
GRAY	732.61 \pm 5.25	774.12 \pm 8.40	705.02 \pm 6.75	737.53 \pm 4.98	771.31 \pm 8.31	714.08 \pm 6.22
WHITE	504.17 \pm 4.19	537.35 \pm 7.02	482.00 \pm 5.19	508.93 \pm 3.97	536.32 \pm 6.94	490.06 \pm 4.78

HC: Healthy controls; ADHD: ADHD patients; M: males; F: females; L: left, R: right; CDN: caudate; NACC: accumbens; AMYG: amygdala; HIP: hippocampus; GP: globus pallidus; PUT: putamen; THA: thalamus; T BRAIN: total of white and gray matter; GRAY: volume of gray matter; WHITE: volume of white matter. Bold indicates results at $p < 0.05$.

In the male ADHD patients, a linear regression analysis showed right caudate volumes were significantly associated with the total number of ADHD symptoms ($\beta = -.35$, $p = .011$). Post-hoc analysis indicated that the association in the right caudate was primarily due to an effect on hyperactive/impulsive ($\beta = -.42$, $p = .002$) rather than inattentive ($\beta = -.15$, $p = .30$) symptoms. In Figure 1, the relationship between right caudate volume and symptom rates is shown.

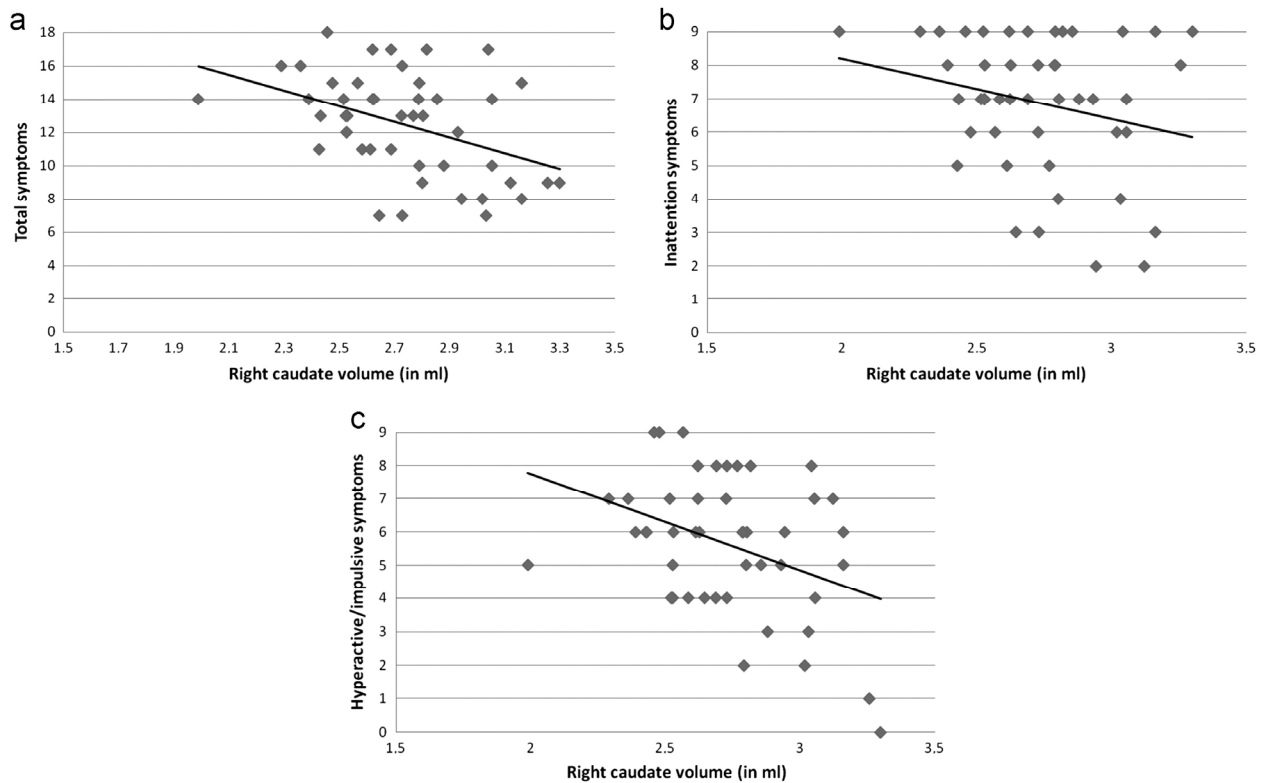


Figure 1. (A–C) Association between the volume of the right caudate in male ADHD patients and number of total, inattentive and hyperactive/impulsive symptoms on the ADHD-DSM-IV Self-Rating scale. The dots represent individual volumes and the solid line represents the linear fit.

In a more exploratory design, we tested the effect of stimulant treatment. As shown in Table 4, stimulant treatment was associated with volumes of the right amygdala ($F(2, 197) = 3.81, p = .024, p_{adj} = .096$, partial eta squared = .037), left and right caudate (left: $F(2, 197) = 3.28, p = .040, p_{adj} = .128$, partial eta squared = .031; right: $F(2, 197) = 3.13, p = .046, p_{adj} = .122$, partial eta squared = .031), left globus pallidus ($F(2, 197) = 3.84, p = .023, p_{adj} = .122$, partial eta squared = .037), right putamen ($F(2, 197) = 4.28, p = .015, p_{adj} = .120$, partial eta squared = .041) and right hippocampus ($F(2, 197) = 6.31, p = .002, p_{adj} = .042$, partial eta squared = .060). The effect on right hippocampus volume survived multiple comparisons correction. Post-hoc tests revealed that patients using stimulant treatment had a smaller right hippocampus volume compared to medication-naïve patients ($p = .001$) and controls ($p = .016$). A linear regression showed that right hippocampus volume was not associated with duration of stimulant use ($\beta = .09, p = .333$).

Table 4. Bilateral and total volumes (in ml) \pm standard error for healthy comparison subjects (HC), stimulant-naïve ADHD patients (ADHD naïve: medication for ≤ 1 month) and stimulant-medicated patients (ADHD medicated: stimulant treatment for > 1 month) based on estimated marginal means and controlled for age, gender and total brain volume

	HC (N = 107)	ADHD naïve (N = 16)	ADHD stimulant-medicated (N = 82)
L ACC	0.35 \pm 0.01	0.37 \pm 0.01	0.34 \pm 0.01
R ACC	0.37 \pm 0.01	0.37 \pm 0.01	0.36 \pm 0.01
L AMY	1.12 \pm 0.01	1.14 \pm 0.04	1.12 \pm 0.02
R AMY	1.10 \pm 0.01	1.20 \pm 0.04	1.10 \pm 0.02
L CDN	2.61 \pm 0.03	2.69 \pm 0.07	2.53 \pm 0.03
R CDN	2.71 \pm 0.03	2.75 \pm 0.07	2.62 \pm 0.03
L HIPP	2.78 \pm 0.03	2.82 \pm 0.07	2.75 \pm 0.03
R HIPP	2.70 \pm 0.02^a	2.83 \pm 0.06^a	2.61 \pm 0.03^a
L GP	0.96 \pm 0.01	1.01 \pm 0.02	0.95 \pm 0.01
R GP	0.93 \pm 0.01	0.96 \pm 0.02	0.92 \pm 0.01
L PUT	3.03 \pm 0.03	3.14 \pm 0.07	2.99 \pm 0.03
R PUT	3.09 \pm 0.03	3.17 \pm 0.08	2.97 \pm 0.04
L THA	5.45 \pm 0.03	5.55 \pm 0.08	5.44 \pm 0.04
R THA	5.78 \pm 0.03	5.90 \pm 0.09	5.69 \pm 0.04
GRAY	738.13 \pm 4.17	744.08 \pm 10.75	741.14 \pm 4.94
WHITE	508.42 \pm 3.29	509.17 \pm 8.49	510.79 \pm 3.90

HC: Healthy controls; ADHD: ADHD patients; L: left, R: right; CDN: caudate; NACC: accumbens; AMYG: amygdala; HIPP: hippocampus; GP: globus pallidus; PUT: putamen; THA: thalamus; T BRAIN: total of white and gray matter; GRAY: volume of gray matter; WHITE: volume of white matter. Bold indicates results at $p < 0.05$.

^a Indicated significance after multiple testing.

As shown in Table 1, 46% of the ADHD patients had had one or more depressive episodes in the past. A comparison between ADHD patients with and without a history of MDD revealed significantly smaller left hippocampus volume in ADHD patients with at least one past depressive episode ($F(1, 108) = 5.21$, $p_{\text{one-sided}} = 0.012$, partial eta squared = 0.043), but no effects on amygdala volumes (Table 5).

Table 5. Bilateral and total hippocampus and amygdala volumes (in ml) \pm standard error for ADHD patients with no MDD history (ADHD -) and ADHD patients remitted from one or more episodes of MDD (ADHD +) based on estimated marginal means and controlled for age, gender and total brain volume

	ADHD depression – (N = 54)	ADHD depression + (N = 53)
L HIPP	2.81 \pm 0.03	2.71 \pm 0.04
R HIPP	2.70 \pm 0.03	2.62 \pm 0.04
L AMYG	1.12 \pm 0.02	1.10 \pm 0.02
R AMYG	1.13 \pm 0.02	1.09 \pm 0.02

ADHD depression -: ADHD patients with no MDD history; ADHD depression +: ADHD patients remitted from one or more MDD; M L: left, R: right; HIPP: hippocampus. Bold indicates results at $p < 0.05$.

Discussion

In the present study, we investigated total, subcortical and hippocampal brain volumes in a sample of 119 adult ADHD patients and 107 healthy comparison subjects. The analyses of regional brain volumes were all adjusted for total brain volume. Our hypothesis of smaller caudate volume in adult ADHD was confirmed, though the right caudate volume was only smaller (relative to brain size) in male patients. This gender effect was consistent with our hypothesis that structural differences are less pronounced in females than in males. We demonstrated in addition, that the reduction in the caudate was correlated with severity of the illness, with a smaller caudate being associated with more ADHD symptoms (primarily hyperactive/impulsive symptoms). We found no reduction of total gray or white matter volume in patients compared to healthy comparison subjects, nor were any of the other subcortical brain volumes or hippocampus volume affected. In the VBM analysis, no additional differences in cortical gray matter volume were observed between adult patients and controls either. Patients using stimulant treatment had a smaller right hippocampus volume compared to medication-naïve patients and controls. ADHD patients with a lifetime history of MDD showed a smaller volume of left hippocampus compared with ADHD patients having no earlier episodes of MDD. Effect sizes (given as partial eta-squared) for the observed effects ranged from 0.043 (for hippocampus volume in depression) to 0.06 (for hippocampus volume in stimulant users) and 0.076 (for caudate nucleus finding in ADHD males), which means that the effect sizes were small to modest.

The fact that only few differences between adult patients and controls were found is in line with hypotheses of developmental delay and normalization of brain structure in adulthood (Shaw et al., 2007; Shaw et al., 2012). Our finding of an absence of total brain (sum of gray and white matter) volume differences is a common finding in ADHD (Biederman et al., 2008; Hesslinger et al., 2002; Perlov et al., 2008; Seidman et al., 2006). The absence of total brain volume in our sample was not reflected by an imbalance between white and gray matter as found by Seidman et al. (2006), nor was it related to an effect of stimulant treatment as reported by Castellanos et al. (2002).

Whereas cortical brain volume seems to normalize, we confirm prior evidence that smaller caudate volume is persistent in ADHD (Almeida Montes et al., 2010; Proal et al., 2011; Seidman et al., 2011). Moreover, the current results extend these studies by showing

that caudate volume phenotype might be a gender-specific effect only observed in males with ADHD. This is in line with a study in children showing smaller basal ganglia volumes only in ADHD boys (Qiu et al., 2009). In addition, our study shows that caudate volume reduction in male patients is associated with ADHD symptoms, with higher hyperactive/impulsive ratings linked to smaller volume in the right caudate. The association with hyperactive/impulsive ratings with caudate volume is consistent with the literature that the caudate is part of the extrapyramidal motor system and plays an important role in locomotor control (Ferris, 1972; Rebec, 2006). Furthermore, a study in ADHD children showed that task performance on a response inhibition task is positively associated with caudate volume (Casey et al., 1997). While there seems to be a link with symptom levels, the link with disease persistence is less clear. The normalization of caudate volume in ADHD by late adolescence as shown in the longitudinal study of (Castellanos et al., 2002) seems to parallel the reported reduction of overt hyperactivity during adolescence (Biederman et al., 2000; Hill and Schoener, 1996). Indeed, we also find that the reduction of caudate volume is correlated with impulsive/hyperactive symptoms in male adult patients, which suggests that persistence of overt hyperactivity might be related to persistent volume reductions of the caudate. On the other hand, Proal et al. (2011) investigated structural differences between ADHD remitters and persisters and found that, among other regions, a reduced right caudate volume was present in patients independent of whether they had persistent ADHD or had remitted. This finding is in line with the hypothesis of Halperin and Schultz (2006) that subcortical dysfunction that manifests early in life, remains static throughout the lifetime, and is not associated with the remission of symptomatology.

Although male and female adults with ADHD have similar phenotypic features in terms of symptom ratings and comorbidity patterns (Biederman et al., 2004), the gender-specific finding for caudate nucleus volume suggests that partially distinct neurobiological deficits underlie ADHD in males and females. At the neurocognitive level, there is evidence that adolescent males with ADHD show more impaired inhibition than female patients (Rucklidge, 2006). Interestingly, the caudate nucleus is involved in impulse inhibition in a sex-specific manner, as shown by a recent study in healthy subjects (Liu et al., 2012). During a stop task, activation of the caudate nucleus and putamen in males was positively correlated with task performance. Females showed a positive correlation in the right inferior temporal gyrus, while activation of the precuneus was negatively correlated with task performance. A better understanding of sex differences in brain anatomy and activity would improve our understanding of the basis for different neurocognitive profiles (such as impulse inhibition) in males and females with ADHD. In that respect, an fMRI study in ADHD looking specifically at gender differences reported that ADHD males, but not ADHD females, showed significantly altered patterns of neural activity during a verbal working memory task (Valera et al., 2010). Importantly, the results from MRI studies in childhood ADHD, which mostly included boys, need to be considered with caution since findings may not generalize to both genders.

The VBM analysis did not pick up the gender-specific effect on right caudate found with FSL FIRST. This is likely due to the fact that the reduction is dispersed across the entire caudate nucleus, with small effects, below detection limit, on individual voxels measured in VBM.

Patients using stimulant treatment had a smaller right hippocampus volume compared to medication-naïve patients and controls. This unexpected finding is not in line with a meta-analysis suggesting that untreated children have additional structural reductions compared with children receiving treatment (Frodl and Skokauskas, 2012). There was no effect of duration of stimulant medication use on the right hippocampus volume, which makes it difficult to draw firm conclusions regarding the effect of long term use of stimulants in adult ADHD.

In the present study we also investigated the effects of the most frequent comorbidity of adult ADHD, i.e. MDD, on brain structure. Observing smaller volumes of total and left hippocampus in ADHD subjects with a history of MDD is in line with well-studied reductions in hippocampal volume in patients with current and recurrent depression (Arnone et al., 2012; Cole et al., 2011; Du et al., 2012; Kempton et al., 2011; Koolschijn et al., 2009; Videbech and Ravnkilde, 2004). Previous findings suggested that depression severity in ADHD adults had no effect on hippocampal volumes (Frodl et al., 2010). In this previous study, depression severity had been measured using a self-report measure of current MDD symptoms, whereas we used a clinical retrospective diagnosis of past MDD episodes. This is a possible explanation for the conflicting results, while there are indications that hippocampal volume may decrease at the greatest rate early after MDD onset (MacQueen et al., 2003). Further studies are clearly needed to fully elucidate the link between MDD and structural brain changes in ADHD.

The results of this study should be considered in the context of some strengths and limitations. Our MRI study sample is the largest one published to date for clinically diagnosed adult ADHD, a clear strength of this work. Because our main hypotheses were based on caudate nucleus, hippocampus and amygdala volumes, we chose to use FSL FIRST, known to be a reliable tool for the automated segmentation of subcortical structures (Morey et al., 2010; Narayana et al., 1988) and successfully used in other studies (De Jong et al., 2008; Franke et al., 2010b; Narayana et al., 1988; Rijpkema et al., 2012; Seror et al., 2010). Certain localized changes in brain structures can also be detected using voxel-based morphometry (VBM), but this method is more sensitive to inaccuracies of tissue-type classification and arbitrary smoothing extents (Patenaude et al., 2011). Due to the poor and variable intensity contrast, VBM might be more prone to registration artifacts in the deep grey matter (Bookstein, 2001). Additionally, Frodl et al. (2012) suggested that changes in smaller regions like the amygdala and hippocampus might be more difficult to detect with VBM when large cluster threshold corrections for the whole brain are used. We performed whole brain VBM analysis in a post-hoc fashion to increase comparability with previous research.

This study was not initially designed to study stimulant medication effects, therefore we were not able to study the effects of different doses of treatment. In addition, treatment histories of patients were solely based on self-report, which may be vulnerable to biases.

In conclusion, the results presented here provide support for the hypothesis that alterations in right caudate volume persist into adulthood in ADHD in males. This gender difference advocates for gender to be taken into account in future neuroimaging studies. Caudate volume correlated with behavioral measures of hyperactivity/impulsivity in the male patients, which may point to different ADHD pathophysiology in men and women. Depression in ADHD was related to hippocampal volume reduction, but it has to be clarified whether effects of current depressive symptoms also exist. Finally, as brain alterations in children and adults with ADHD seem not to be restricted to isolated brain regions in most cases, further studies should also investigate structural and functional connectivity in neural networks in ADHD.

Supplementary information

Supplementary Table 1. Correlation matrix of bilateral and total volumes

	T CDN	L CDN	R CDN	T NACC	L NACC	R NACC	T AMYG	L AMYG	R AMYG	T HIP	L HIP	R HIP	T GP	L GP	R GP	T PUT	L PUT	R PUT	T THA	L THA	R THA
T CDN	1.00	0.92	0.92	0.64	0.55	0.60	0.43	0.37	0.40	0.57	0.51	0.54	0.64	0.62	0.60	0.64	0.64	0.61	0.63	0.61	0.62
L CDN	0.92	1.00	0.71	0.56	0.49	0.52	0.35	0.30	0.33	0.47	0.41	0.47	0.59	0.58	0.53	0.58	0.59	0.54	0.56	0.55	0.55
R CDN	0.92	0.71	1.00	0.63	0.54	0.60	0.44	0.38	0.42	0.57	0.53	0.53	0.60	0.57	0.57	0.60	0.60	0.58	0.60	0.58	0.59
T NACC	0.64	0.56	0.63	1.00	0.90	0.91	0.48	0.42	0.46	0.58	0.52	0.54	0.65	0.62	0.60	0.69	0.69	0.64	0.68	0.64	0.68
L NACC	0.55	0.49	0.54	0.90	1.00	0.64	0.48	0.44	0.44	0.52	0.48	0.47	0.60	0.62	0.53	0.62	0.64	0.56	0.60	0.57	0.60
R NACC	0.60	0.52	0.60	0.91	0.64	1.00	0.39	0.32	0.39	0.53	0.47	0.51	0.57	0.51	0.57	0.63	0.61	0.61	0.63	0.59	0.64
T AMYG	0.43	0.35	0.44	0.48	0.48	0.39	1.00	0.91	0.91	0.61	0.57	0.56	0.71	0.71	0.64	0.57	0.58	0.53	0.52	0.51	0.49
L AMYG	0.37	0.30	0.38	0.42	0.44	0.32	0.91	1.00	0.65	0.54	0.53	0.47	0.63	0.66	0.54	0.49	0.50	0.45	0.44	0.45	0.42
R AMYG	0.40	0.33	0.42	0.46	0.44	0.39	0.91	0.65	1.00	0.57	0.51	0.55	0.67	0.63	0.63	0.54	0.55	0.51	0.49	0.48	0.48
T HIP	0.57	0.47	0.57	0.58	0.52	0.53	0.61	0.54	0.57	1.00	0.92	0.92	0.68	0.68	0.61	0.59	0.60	0.54	0.67	0.63	0.67
L HIP	0.51	0.41	0.53	0.52	0.48	0.47	0.57	0.53	0.51	0.92	1.00	0.70	0.61	0.62	0.54	0.52	0.53	0.48	0.61	0.60	0.58
R HIP	0.54	0.47	0.53	0.54	0.47	0.51	0.56	0.47	0.55	0.92	0.70	1.00	0.64	0.64	0.58	0.56	0.57	0.53	0.63	0.56	0.66
T GP	0.64	0.59	0.60	0.65	0.60	0.57	0.71	0.63	0.67	0.68	0.61	0.64	1.00	0.95	0.95	0.77	0.78	0.71	0.69	0.68	0.67
L GP	0.62	0.58	0.57	0.62	0.62	0.51	0.71	0.66	0.63	0.68	0.62	0.64	0.95	1.00	0.79	0.73	0.77	0.64	0.69	0.68	0.67
R GP	0.60	0.53	0.57	0.60	0.53	0.57	0.64	0.54	0.63	0.61	0.54	0.58	0.95	0.79	1.00	0.73	0.70	0.71	0.62	0.62	0.60
T PUT	0.64	0.58	0.60	0.69	0.62	0.63	0.57	0.49	0.54	0.59	0.52	0.56	0.77	0.73	0.73	1.00	0.97	0.97	0.65	0.62	0.65
L PUT	0.64	0.59	0.60	0.69	0.64	0.61	0.58	0.50	0.55	0.60	0.53	0.57	0.78	0.77	0.70	0.97	1.00	0.89	0.66	0.63	0.66
R PUT	0.61	0.54	0.58	0.64	0.56	0.61	0.53	0.45	0.51	0.54	0.48	0.53	0.71	0.64	0.71	0.97	0.89	1.00	0.61	0.58	0.60
T THA	0.63	0.56	0.60	0.68	0.60	0.63	0.52	0.44	0.49	0.67	0.61	0.63	0.69	0.69	0.62	0.65	0.66	0.61	1.00	0.97	0.98
L THA	0.61	0.55	0.58	0.64	0.57	0.59	0.51	0.45	0.48	0.63	0.60	0.56	0.68	0.68	0.62	0.62	0.63	0.58	0.97	1.00	0.91
R THA	0.62	0.55	0.59	0.68	0.60	0.64	0.49	0.42	0.48	0.67	0.58	0.66	0.67	0.67	0.60	0.65	0.66	0.60	0.98	0.91	1.00

L: left, R: right; CDN: caudate; NACC: accumbens; AMYG: amygdala; HIP: hippocampus; GP: globus pallidus; PUT: putamen; THA: thalamus.

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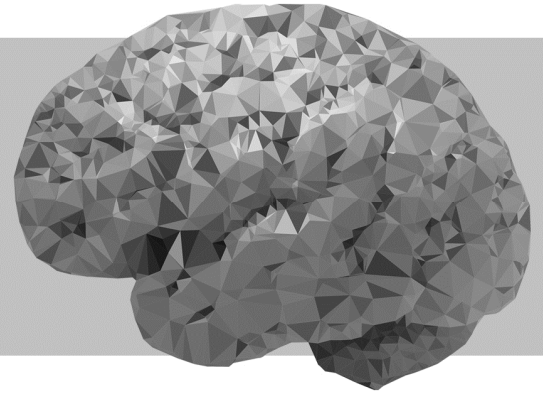
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Deviant white matter structure in adults with ADHD points to aberrant myelination and affects neuropsychological performance

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Abstract

Attention-deficit/hyperactivity disorder (ADHD) in childhood is characterized by gray and white matter abnormalities in several brain areas. Considerably less is known about white matter microstructure in adults with ADHD and its relation with clinical symptoms and cognitive performance. In 107 adult ADHD patients and 109 gender-, age- and IQ-matched controls, we used diffusion tensor imaging (DTI) with tract-based spatial statistics (TBSS) to investigate whole-skeleton changes of fractional anisotropy (FA) and mean, axial, and radial diffusivity (MD, AD, RD). Additionally, we studied the relation of FA and MD values with symptom severity and cognitive performance on tasks measuring working memory, attention, inhibition, and delay discounting. In comparison to controls, participants with ADHD showed reduced FA in corpus callosum, bilateral corona radiata, and thalamic radiation. Higher MD and RD were found in overlapping and even more widespread areas in both hemispheres, also encompassing internal and external capsule, sagittal stratum, fornix, and superior lateral fasciculus. Values of FA and MD were not associated with symptom severity. However, within some white matter clusters that distinguished patients from controls, worse inhibition performance was associated with reduced FA and more impulsive decision making was associated with increased MD. This study shows widespread differences in white matter integrity between adults with persistent ADHD and healthy individuals. Changes in RD suggest aberrant myelination as a pathophysiological factor in persistent ADHD. The microstructural differences in adult ADHD may contribute to poor inhibition and greater impulsivity but appear to be independent of disease severity.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood psychiatric disorder with an estimated prevalence around 5.3% in childhood that persists through adolescence reaching a prevalence of up to 4.9% in adults (Simon et al., 2009). ADHD is associated with global and regional brain volume reductions. Meta-analytic findings show reductions in total cerebral volume, in frontal lobes, cingulate cortex, and corpus callosum; in addition, robust evidence exists for decreased gray matter volume in subcortical areas (Ellison-Wright et al., 2008; Frodl and Skokauskas, 2012; Nakao et al., 2011; Valera et al., 2007). Differences in subcortical structures such as the putamen and caudate seem to disappear with increasing age (Castellanos et al., 2002; Nakao et al., 2011). Moreover, longitudinal studies show a delay in the age by which peak cortical thickness is reached in ADHD patients (Shaw et al., 2007), which has led to the suggesting that ADHD may be the outcome of a maturational lag that eventually normalizes (Rubia, 2007). More recent results of longitudinal studies indicate, however, that reductions in basal ganglia, which were detected in childhood, persisted into adolescence (Shaw et al., 2014). Cross-sectional studies in adults with ADHD also point to persistent gray matter reductions in subcortical volumes (Frodl et al., 2010; Onnink et al., 2014; Proal et al., 2011; Seidman et al., 2011) as well as in cortical areas (Ahrendts et al., 2011; Amico et al., 2011; Biederman et al., 2008; Makris et al., 2007; Seidman et al., 2011; Seidman et al., 2006), and in cerebellar regions (Proal et al., 2011; Seidman et al., 2011).

Over the last decade, the focus of neuroimaging research has widened from studies of regional volume alterations to studies of altered white matter connections within and among several neural networks (Konrad and Eickhoff, 2010). Advances in diffusion tensor imaging (DTI) allowed non-invasive investigation of white matter tracts connecting cortical and subcortical regions (Thomason and Thompson, 2011). DTI probes both the microstructural organization and the myelination of white matter through measuring the diffusion of water molecules in the tissue (Beaulieu, 2002; Le Bihan et al., 2001). Commonly used parameters are fractional anisotropy (FA) and mean diffusivity (MD), which reflect the preferential directionality of water diffusion along white matter tracts and the magnitude of diffusion, respectively (Le Bihan et al., 2001). Although decreased FA is a characteristic of impaired white matter integrity, its exact neurobiological meaning is not fully understood (Beaulieu, 2002).

Impaired white matter integrity has been found in numerous psychiatric disorders including major depressive disorder (Korgaonkar et al., 2011), bipolar disorder (Barysheva et al., 2013), schizophrenia (Mandl et al., 2013) and ADHD. A meta-analysis in children, adolescents, and adults with ADHD provided evidence of microstructural abnormalities in areas such as the anterior corona radiata (ACR), forceps minor, bilateral internal capsule, and cerebellum (van Ewijk et al., 2012). This meta-analysis only included hypothesis-free whole-brain voxelwise (VBA) approaches and could not provide directionality of findings (e.g., higher or lower FA in ADHD). Hypothesis-driven region of interest (ROI) studies reported that ADHD is in general associated with lower FA in the corpus callosum (Cao et al.,

2010), cerebellum (Bechtel et al., 2009), and in several fronto-striatal tracts (Hamilton et al., 2008; Pavuluri et al., 2009; Shang et al., 2013; Wu et al., 2014). Some studies revealed that ADHD patients had *higher* FA (de Zeeuw et al., 2012; Silk et al., 2009; Tamm et al., 2012) in fronto-striatal regions when compared with healthy controls. A recent study found clusters of decreased FA and MD in most of the major white matter tracts and concluded that white matter alterations are a wide-ranging rather than localized feature in children and adolescents with ADHD (van Ewijk et al., 2014). Analyses using graph theory in combination with whole-brain DTI (e.g., brain connectomics) revealed similarly that, in children and adolescents with ADHD, decreased white matter connectivity in fronto-striatal circuits extended to a larger brain network which encompassed additional cortico-cortical, subcortical, and cerebellar circuits (Hong et al., 2014). The few available DTI studies of adult ADHD patients to date showed decreased FA in tracts such as the cingulum bundle (Makris et al., 2008), the inferior longitudinal fasciculus (ILF) (Konrad et al., 2012), the superior longitudinal fasciculus (SLF) (Cortese et al., 2013; Makris et al., 2008), and the corpus callosum (Dramsdaahl et al., 2012). Although the current ADHD literature lacks longitudinal DTI studies, decreased FA has been reported in persistent *and* remitted adult patients with ADHD in comparison with healthy controls. These persistent findings were observed in areas including the corona radiata, sagittal stratum, the retrolenticular internal capsule, and the SLF (Cortese et al., 2013). Conversely, another study found that remitted adult patients did not differ significantly from controls, while patients with persistent ADHD had decreased FA in the uncinate and inferior fronto-occipital fasciculi (Shaw et al., 2015).

Decreased FA is typically accompanied by increased MD values in studies of ADHD. Increased MD is related with decreased cellular density (Alexander et al., 2007) and may reflect abnormalities in ADHD more sensitively than FA (de Luis-Garcia et al., 2015; Lawrence et al., 2013). Moreover, decreased FA might result from increased radial diffusivity (RD) and/or reduced axial diffusivity (AD) (Alexander et al., 2007). While the biological correlates of those measures are not yet entirely clarified, decreases in AD are currently thought to indicate axonal damage or degeneration, and increases in RD with minimal changes in AD are thought to indicate increased freedom of cross-fibre diffusion and possibly decreased myelination (Alexander et al., 2007; Song et al., 2002). Reporting changes in RD and AD could potentially help elucidate the FA findings in studies of ADHD. In the ADHD childhood literature, reports on RD have shown the entire range from increased RD (Helpern et al., 2011; Nagel et al., 2011) to decreased RD (Silk et al., 2009), and one study reported no change in RD (Tamm et al., 2012). Increased AD (together with an increased FA) has been reported in two childhood studies (Silk et al., 2009; Tamm et al., 2012). A recent study in adult ADHD patients suggested that reductions of FA were driven by changes in RD rather than AD (Shaw et al., 2015).

In addition to case-control comparisons, some studies investigated the behavioral implications of changed white matter variation in patients with ADHD by looking at its association with clinical symptoms or cognitive measures. Although findings in the ADHD literature are heterogeneous and complex, most studies have found that increasing

symptom severity was associated with decreased FA (Ashtari et al., 2005; Nagel et al., 2011; Shang et al., 2013), but also with higher FA (Peterson et al., 2011; van Ewijk et al., 2014). In an adult ADHD study, attentional performance correlated with FA and MD in the right SLF, and measures of impulsivity correlated with FA in right orbitofrontal fiber tracts (Konrad et al., 2010).

Taken together, there is strong evidence for wide-spread white matter differences in ADHD patients compared to controls, and these may be related to ADHD symptomatology and cognitive functioning. Findings in the ADHD literature differ in precise location and directionality, which makes comparison of studies difficult. This is likely due to differences in sample characteristics (e.g., gender, age ranges), small sample sizes, and methodological differences (e.g., use of VBA versus ROI approaches). Relative to childhood and adolescent ADHD studies, there are few DTI studies in adult patients, and those are hampered by small sample sizes and by the use of ROIs instead of whole-brain approaches (except for the study by Cortese and coworkers (2013)). In adult ADHD, only few studies investigated AD and RD (Shaw et al., 2015), associations with ADHD symptomatology (Dramsdaahl et al., 2012; Shaw et al., 2015), and cognitive performance (Konrad et al., 2012). Therefore, an overall picture of white matter pathology in adult ADHD is currently lacking.

In this study, we used DTI to comprehensively compare white matter variation in adults with ADHD and healthy controls. We investigated values of FA, MD, AD, and RD using tract-based spatial statistics (TBSS), which is a whole-skeleton voxel-by-voxel analysis (Smith et al., 2006). Within the ADHD group, we investigated associations of FA and MD with clinical symptom scores and cognitive measures. These cognitive measures were selected to cover prominent cognitive domains commonly affected in adults with ADHD (e.g., working memory, attention, inhibition, and delay discounting/impulsivity). Based on the current literature, we expected to find (a) widespread decreases of FA and increases of MD and RD in ADHD, and (b) associations of FA with symptom severity and cognitive performance.

Materials and Methods

Subjects and procedure

In total, 216 individuals (107 patients with persistent ADHD, 109 healthy controls) from the Dutch cohort of the International Multicentre persistent ADHD CollaboraTion (IMpACT) (Franke et al., 2010) participated in this study. The patients and an age-, gender-, and IQ-matched group of healthy controls were recruited through the Department of Psychiatry of the Radboud university medical center and through advertisements.

Patients were included if they met DSM-IV-TR criteria for ADHD in childhood as well as adulthood, as assessed by a psychiatrist. At the time of inclusion into the study, participants were assessed using the Diagnostic Interview for Adult ADHD (DIVA) (Kooij, 2010). This interview focuses on the 18 DSM symptoms of ADHD and uses concrete and realistic examples to thoroughly investigate, whether a symptom is currently present or was present in childhood. In order to obtain information about ADHD symptoms and impairment in childhood, additional information was acquired from parent and school reports, whenever

possible. The Structured Clinical Interview for DSM-IV (SCID-I & SCID-II) was used for comorbidity assessment (see Table 1). Assessments were carried out by trained professionals (psychiatrists or psychologists). In addition, a quantitative measure of clinical symptoms was obtained using the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

Table 1 Demographic, clinical, and cognitive characteristics of ADHD patients and healthy controls (HC)

	ADHD (N = 107)	HC (N = 109)	Test of significance
Gender (males/females)	41/66	47/62	$\chi^2 = 0.51, p = .47$
Age (years)	35.00 ± 10.30	36.08 ± 10.97	$t(1,214) = 0.74, p = .46$
IQ ^a	108.13 ± 14.43	110.97 ± 15.36	$t(1, 214) = 1.40, p = .16$
Inattention symptoms ^b	7.27 ± 1.56	0.59 ± 1.29	$t(1, 214) = -34.48, p < .0001$
Hyperactivity/Impulsivity symptoms ^b	5.65 ± 2.36	0.59 ± 1.12	$t(1, 214) = -19.96, p < .0001$
Digit span ^c	6.77 ± 2.2	7.53 ± 2.38	$F(1,208) = 6.56, p < .01$
SAD ^d	3.53 ± 0.26	3.42 ± 0.19	$F(1,202) = 11.94, p < .001$
SART ^e	11.02 ± 4.76	9.29 ± 5.04	$F(1,187) = 5.31, p = .02$
Delay discounting ^f	0.038 ± 0.064	0.010 ± 0.015	$F(1,187) = 16.31, p < .0001$
DTI acquisition protocol ^g	35 (33%)	23 (21%)	$\chi^2 = 3.71, p = .05$
One or more depressive episode(s) (remitted) ^h	52 (57%)	12 (11%)	
Anxiety disorder (remitted) ^h	22 (23%)	6 (6%)	
Substance use disorder (remitted) ^h	21 (20%)	6 (6%)	
Borderline Personality D ^h	10 (9%)	-	
Medication-naïve	20 (19%)	-	
On stimulant medication	64 (60%)	-	
Medication in the past	14 (13%)	-	
On atomoxetine	9 (8%)	-	

Demographic information representing means \pm standard deviations or percentage per group.

^a Prorated from Block Design and Vocabulary of WAIS-III-R.

^b As measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^c Digit Span raw backwards score (working memory).

^d Errors Sustained Attention Dots (SAD) task (attention).

^e Commission errors Sustained Attention to Response Task (SART) (inhibition).

^f Score on Delay Discounting task (impulsivity).

^g First version of DTI acquisition protocol.

^h As measured by the Structured Clinical Interview for DSM-IV for axis I (Groenestijn et al., 1999) and axis II (Weertman et al., 2000) disorders.

Exclusion criteria for participants were psychosis, alcohol or substance use disorder in the last 6 months, current major depression, full-scale IQ estimate <70 (assessed using the Wechsler Adult Intelligence Scale-III), neurological disorders, sensorimotor disabilities, non-Caucasian ethnicity, and medication use other than psychostimulants, atomoxetine, or bupropion. An additional exclusion criterion for the healthy control subjects was a current neurological or psychiatric disorder according to SCID-I and SCID-II. This study was approved

by the regional ethics committee (Centrale Commissie Mensgebonden Onderzoek: CMO Regio Arnhem – Nijmegen; Protocol number III.04.0403). Written informed consent was obtained from all participants.

Acquisition of diffusion-weighted images

Whole-brain imaging was performed with a 1.5 Tesla MR scanner (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) and a standard 8 channel head coil. A 3D T1-weighted MPRAGE anatomical scan was obtained from each subject (Repetition Time (TR) = 2730 ms, Echo Time (TE) = 2.95 ms, Inversion Time (TI) = 1000 ms, flip angle = 7°, field of view = 256 x 256 x 176 mm³, voxel size = 1.0 x 1.0 x 1.0 mm³). The T1 images served as high resolution anatomical reference image for diffusion imaging data. Transversely oriented diffusion-weighted images were acquired using a twice-refocused spin-echo-planar-imaging sequence that minimized imaging distortions from eddy currents (Reese et al., 2003). The diffusion imaging data were acquired using two different protocols. Fifty-eight subjects were scanned with the following protocol: TR = 10200 ms, TE = 95 ms, field of view = 320 x 320 x 160 mm³, voxel size = 2.5 x 2.5 x 2.5 mm³, 6/8 partial Fourier. Four images without diffusion-weighting ($b=0$ s/mm²) and 30 images with diffusion-weighting ($b=900$ s/mm², diffusion directions = 34) applied along non-collinear directions were acquired. The remaining 158 subjects were scanned with an improved second protocol, which was implemented to reduce motion artifacts during scanning. Parameters that differed from the first protocol were TR (6700 ms), TE (85 ms), field of view (220 x 220 x 140 mm³), and scans were acquired with full Fourier acquisition, other parameters were unchanged. For each slice, the diffusion-weighting for the 30 images changed to $b=900$ s/mm². Acquisition protocol was included as covariate in all analyses.

Preprocessing and skeletonization of diffusion-weighted images

The diffusion-weighted data was preprocessed using an in-house developed algorithm. In short, the diffusion-weighted images of each subject were realigned on the unweighted image using mutual information routines from SPM8 (Wellcome Trust Center for Neuroimaging). Next, an iteratively reweighted-least-squares algorithm (PATCH) was used to robustly correct for head and cardiac motion artifacts in the diffusion-weighted data (Zwiers, 2010). Using DTIFIT from the FMRIB's Diffusion Toolbox (part of FMRIB's Software Library (FSL)), FA images were created and subsequently fed into the TBSS pipeline (Smith et al., 2006). Here, all individual FA maps were nonlinearly registered to the FMRIB58_FA template using FSL's nonlinear registration tool FNIRT. Then, the nonlinear transforms found in the previous stage were applied to all subjects to bring them into standard Montreal Neurological Institute (MNI) space. A mean FA image was created and thinned to create a mean FA skeleton which represents the centers of all tracts common to the group. A threshold of 0.2 was used to avoid partial voluming effects. Individual FA images were then mapped onto this skeleton resulting in a skeletonized FA image for each individual. Finally,

each participant's FA, MD, AD, and RD image was projected onto this skeleton, and resulting data were used for voxel-wise statistics.

Neuropsychological assessment

Cognitive functioning of participants was assessed by a neuropsychological test battery that was composed to cover multiple cognitive domains earlier found affected in ADHD (Mostert et al., 2015): (i) working memory, assessed via the WAIS-III Digit Span task (Wechsler, 1997); (ii) attention, measured with the response bias variable of the Sustained Attention Dots task (De Sonneville, 1999); (iii) inhibition, tested via the Sustained Attention to Response Task (SART) (Smit et al., 2004); and (iv) delay discounting/impulsivity assessed via the Delay Discounting task (Dom et al., 2006). Assumptions with respect to the residuals were checked and neuropsychological measures were log-transformed if necessary to achieve a normal distribution. Outliers were defined as having a score more extreme than 4-times the standard deviation above or below the mean per group. Details of task and outcome measures are described in Supplementary Table 1.

Statistical analysis

First, we performed a between-subject whole-skeleton voxel-wise analysis using TBSS, in which we compared patients to control subjects on values of FA, MD, AD, and RD. In all analyses, gender, age, and scan acquisition protocol were included in the model as nuisance regressors. Threshold-free cluster enhancement (TFCE) was applied to obtain cluster-wise statistics corrected for multiple comparisons. Briefly, this method transforms local T-statistics into TFCE statistics that reflect both the size of the local effect (or “height”) and the cluster extent (Smith and Nichols, 2009). With the obtained TFCE maps, “randomize” then calculates a p -value (p -corrected) for each voxel, corrected for whole-skeleton family-wise error (FWE) rate via permutation testing (5000 permutations). The TFCE-corrected p -value maps were thresholded at $P_{FWE} = 0.05$, and we report regions that contained clusters of at least ten contiguous suprathreshold voxels. Significant results were localized to anatomical locations using the Johns Hopkins University (JHU) – ICBM-DTI-81 white matter labels atlas (Mori et al., 2008) and the white matter tractography atlas (Hua et al., 2008). To estimate the effect size of significant clusters, spatially averaged scores were calculated from significant clusters for each subject, and subsequently partial eta-squared was calculated using SPSS version 21 (IBM, Chicago, IL).

Secondly, within the ADHD group we performed two whole-skeleton regression analyses in TBSS similar to van Ewijk et al. (2014). This analysis was performed using self-reported symptom counts on both dimensions (inattention and hyperactivity/impulsivity) as two separate predictors. Gender, age, and scan acquisition protocol were included in the model as nuisance regressors. The TFCE-corrected p -value maps were thresholded at $P_{FWE} = 0.05$. In addition, two analyses were performed in the ADHD group to further investigate significant between-group findings from the first TBSS analysis in an ROI approach for their link with symptom severity. From each significant FA and MD cluster, an ROI mask was

created and was then back-projected to the original images of each individual; subsequently, spatially averaged FA and MD values were obtained. Partial correlation analyses were performed (in SPSS) to identify correlations between the extracted average of FA and MD for each cluster and self-reported symptom count on both dimensions, adjusting for gender, age, and scan acquisition protocol.

Third, similar partial correlation analyses as listed above were performed (in SPSS) for the extracted average of FA and MD (for each cluster) and cognitive measures (working memory, attention, inhibition, delay discounting/impulsivity), adjusting for gender, age, and scan acquisition protocol. These partial correlation analyses were performed in the whole group. Post-hoc analyses were carried out for significant findings, in which the ADHD and control group were tested separately to explore potential group-specific effects. For the two latter analyses, Bonferroni correction was used and the p -value of 0.05 was divided by the number of significant FA and MD clusters and multiplied by two for the analysis with symptoms dimensions and multiplied by four for the analysis with the four cognitive measures.

Lastly, to explore whether stimulant medication or a history of comorbid major depressive disorder, the most frequent comorbidity of ADHD in our cohort, confounded our between-group results, general linear models (GLM) were used (in SPSS). The extracted mean values from the significant between-group FA, MD, RD, and AD clusters were included as dependent factors. For the GLM of medication, healthy controls ($N=109$), medication-naïve patients ($N=20$), and patients using stimulant treatment ($N=64$) were added as between subject factors. For the GLM of depression history, healthy controls with no history of depressive episodes ($N=95$), ADHD patients with no history of depressive episodes ($N=43$), and ADHD patients with one or more episodes in the past ($N=52$) were added as between subject factors. Post-hoc analyses were performed using Fisher's least significant difference (LSD).

Results

Demographic, clinical and cognitive measures

Across the two groups, there were no significant differences in age of participants or in gender distribution. As expected, patients with ADHD scored significantly higher on ADHD symptom counts and significantly worse on cognitive measures, compared to the control group. The details are summarised in Table 1.

Between-group TBSS analysis of white matter microstructure

The whole-skeleton voxel-based between-group analysis with TBSS identified several clusters of decreased FA and increased MD and RD in the ADHD group when compared to the control group (Table 2, Figure 1). No regions of increased FA or reduced MD or RD were observed, and no differences were observed for AD. For FA, differences between patients and controls were located in the body and splenium of the corpus callosum, anterior and superior corona radiata, posterior thalamic radiation, and tapetum. For MD and RD,

overlapping regions were found, although case-control differences were even more widespread in both right and left hemisphere, also encompassing internal and external capsule, sagittal stratum, fornix, and SLF. The same pattern of results was observed when the analysis for FA was limited to the single scan acquisition protocol on which most scans were performed (N=158; Supplementary Table 4).

AD and RD are derived from three quantitative indices (i.e. eigenvalues— λ_1 , λ_2 , λ_3) that index tissue structure based on water molecule displacement. The first eigenvalue (λ_1) measures AD, while RD is the average of the second (λ_2) and third (λ_3) eigenvalue. As a consequence, the signal-to-noise ratio of RD is $\sqrt{2}$ (the square root of 2) times higher than that of AD, which results in less power (through higher standard errors) to detect AD differences than RD differences. The absence of significant AD clusters in conjunction with positive RD clusters in our sample might thus have been due to power differences. In order to clarify this, we extracted mean AD and RD with standard errors from the significant between-group FA clusters. As expected, we found that the standard error for AD (3.14×10^{-6}) was 1.27 times higher than the one for RD (2.46×10^{-6}). The mean RD values significantly higher in the ADHD group compared to controls, ($F(1, 211) = 18.880$, $p = .00002$), while mean AD values were not ($F(1, 211) = .739$, $p = .391$). Furthermore, when we decomposed RD by extracting mean λ_2 and λ_3 from the significant between-group FA clusters and compared them between patients and controls, we found significant differences between patients and controls for both λ_2 ($F(1, 211) = 18.901$, $p = .00002$) and λ_3 ($F(1, 211) = 16.719$, $p = .00006$).

Table 2. Clusters showing significant differences in Fractional Anisotropy (FA), Mean Diffusivity (MD), and Radial Diffusivity (RD) between ADHD patients (N =107) and healthy controls (N = 109)

Cluster	White matter tracts overlapping with the clusters (size of overlap in >10 voxels) ^a	Size (voxels) ^b	MNI coordinates (x;y;z)	Partial eta ^{2c}	p ^d
<i>Clusters with significantly lower FA in ADHD patients</i>					
1	Body and splenium of CC	453	-1;-26;23	.080	.042
2	Splenium of CC, SCR (R), PCR (R)	141	24;-35;28	.062	.046
3	Body of CC, SCR (R)	140	17;-24;33	.068	.040
4	Splenium of CC	56	16;-36;29	.055	.049
5	PCR (R)	32	18;7;34	.048	.049
6	PTR (R), Tapetum (R)	16	30;-51;15	.037	.049
<i>Clusters with significantly higher MD in ADHD patients</i>					
1	Body, splenium and genu of CC, EC (L+R), ACR (L+R), PCR (L+R), SCR (L), posterior limb of IC (L+R), retrolenticular part of IC (L+R), anterior limb of IC (L), sagittal stratum (R), cingulum, fornix (cres) / stria terminalis (L), CP (L), PTR (L+R), SFOF (L), fornix (cres) / Stria terminalis (R)	6763	37;-31;5	.153	.037
2	SLF (L)	407	-49;-38;12	.126	.042
3	EC (L)	40	-35;-9;-11	.086	.047
4	Sagittal stratum (L)	36	-42;-13;-15	.076	.048
5	Sagittal stratum (L)	16	-40;-23;-7	.059	.049
<i>Clusters with significantly higher RD in ADHD patients</i>					
1	Body, splenium and genu of CC, ACR (L+R), SCR (L+R), PCR (L+R), PTR (L+R), EC (L), retrolenticular part of IC (L+R), anterior limb of IC (L), posterior limb of IC (L+R), fornix (cres) / stria terminalis (L), sagittal stratum (L), SFOF (L), UF (L), SLF (L+R)	8411	2;-27;23	.133	.027
2	Sagittal stratum (R), EC (R), Fornix (cres) / Stria terminalis (R)	454	35;-13;-12	.099	.045
3	SLF (L)	386	-56;-24;5	.122	.044
4	SLF (L)	119	-18;28;30	.049	.048

CC corpus callosum, ACR anterior corona radiata, FA fractional anisotropy, MD mean diffusivity, PCR posterior corona radiata, RD radial anisotropy, SCR superior corona radiata, RPIC retrolenticular part of IC, PTR posterior thalamic radiation (include optic radiation), PLIC posterior limb of IC, SLF superior longitudinal fasciculus, IC internal capsule, EC external capsule, SFOF superior fronto-occipital fasciculus, UF uncinate fasciculus.

^a White matter tracts as defined with the Johns Hopkins University White Matter Label Atlas.

^b Cluster size > 10 voxels.

^c Partial eta squared based on mean FA, MD and RD of the cluster.

^d $P < .05$, FWE-corrected, controlling for gender, age and scan acquisition protocol.

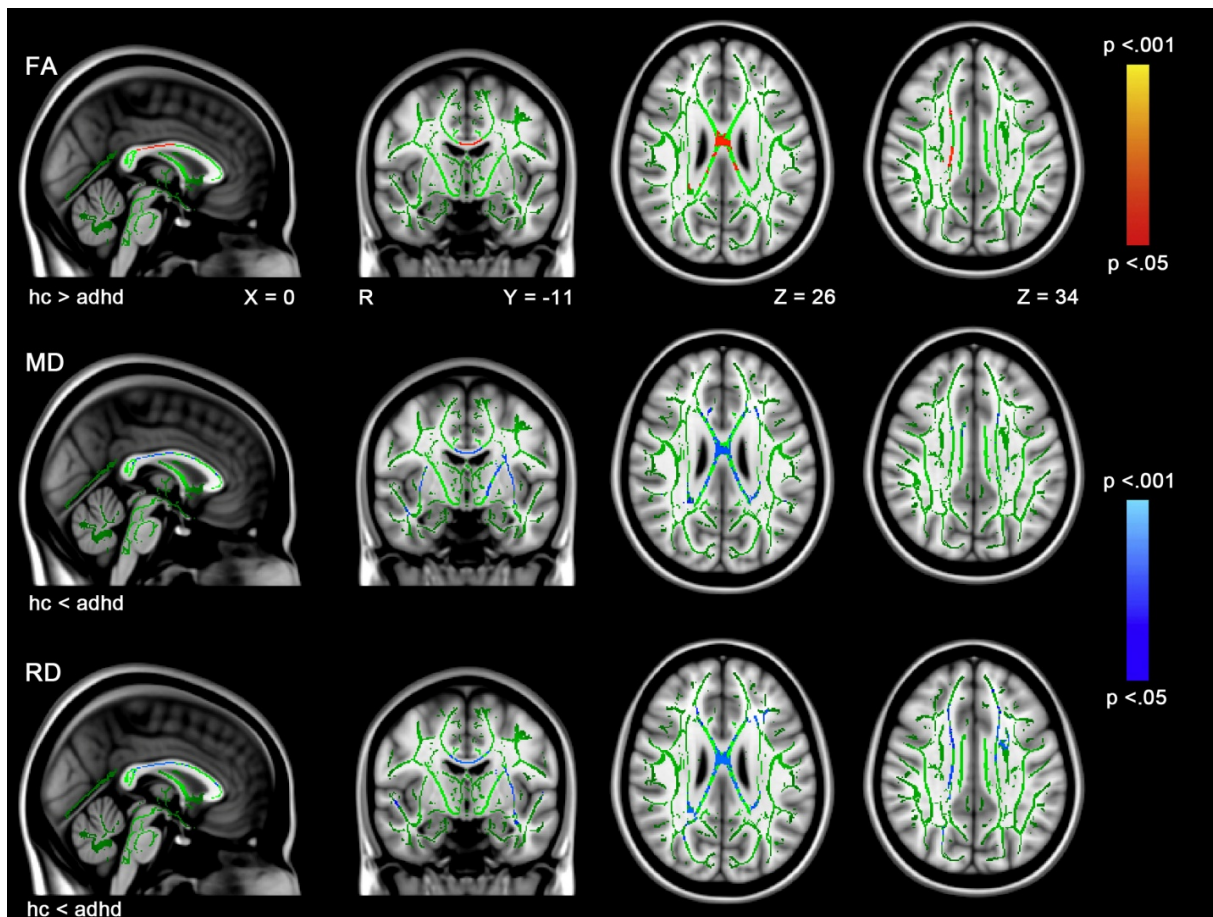


Figure 1. Results from the tract-based spatial statistics (TBSS) analyses displayed on the MNI152 brain. Hot colors represent increased values, and cool colors represent decreased values. Decreased fractional anisotropy (FA), increased mean diffusivity (MD) and radial diffusivity (RD) are shown in individuals with ADHD compared to controls (threshold-free cluster enhancement, $p < .05$, corrected).

Association test of FA and MD with symptom scores in patients with ADHD

The whole-skeleton voxel-based regressions with TBSS in the patients showed that both ADHD symptoms domains (inattention and hyperactivity/impulsivity) were not associated with FA or with MD. Furthermore, the partial correlation analyses of mean values of the significant between-group FA and MD clusters with either symptom domain did not show any significant correlations ($p_{adj} > .05$).

Association of FA and MD with cognitive measures in patients and controls

In the whole group, the partial correlation analyses showed that inhibition performance was negatively correlated with FA in cluster 4 ($r = -.265$; $p = .0001$), such that worse inhibition (i.e., more commission errors on the SART) was linked to lower FA. The delay discounting score was positively correlated with MD in cluster 1 ($r = .242$; $p = .0008$), such that steeper discounting on the Delay Discounting task (i.e., higher impulsivity) was linked to higher MD. To further explore which group contributed to the effects reported above, post-hoc analyses in the patients and in the control group separately revealed that the correlation with inhibition performance was predominantly present in the control group ($r = -.288$; $p = .004$) and did not reach significance in the ADHD patient group ($r = -.179$; $p = .099$). Steeper delay

discounting was correlated with MD only in the ADHD patient group ($r = .283$; $p = .009$) and not among controls ($r = .032$; $p = .750$) (Figure 2). There were no significant results for working memory or attentional performance (Table 3).

Table 3. Partial correlations between mean Fractional Anisotropy (FA) and Mean Diffusivity (MD) of between-subject clusters and cognitive measures controlling for age, gender, and scan protocol

	Digit span ^a 109 HC/103 ADHD (N)	SAD ^b 106 HC/100 ADHD (N)	SART ^c 100 HC/90 ADHD (N)	Delay discounting ^d 102 HC/88 ADHD (N)
FA cluster 1	.136*	-.105	-.223*	-.140
FA cluster 2	.039	-.127	-.068	-.051
FA cluster 3	-.066	-.090	-.036	-.133
FA cluster 4	.063	-.088	-.265**	-.213*
FA cluster 5	.1	-.054	-.101	-.043
FA cluster 6	.163*	-.018	-.054	-.175
MD cluster 1	-.054	.201*	.160*	.242**
MD cluster 2	-.063	.214*	.072	.121
MD cluster 3	-.108	.135	.094	.182*
MD cluster 4	-.099	.144*	.071	.142
MD cluster 5	-.033	.118	.033	.185*

^a Digit Span raw backwards score (working memory)

^b Errors Sustained Attention Dots (SAD) task (attention)

^c Commission errors Sustained Attention to Response Task (SART) (inhibition)

^d Score on Delay Discounting task (impulsivity)

* Indicates an uncorrected significance of $p < .05$

** indicates a corrected significance of $p < .001$

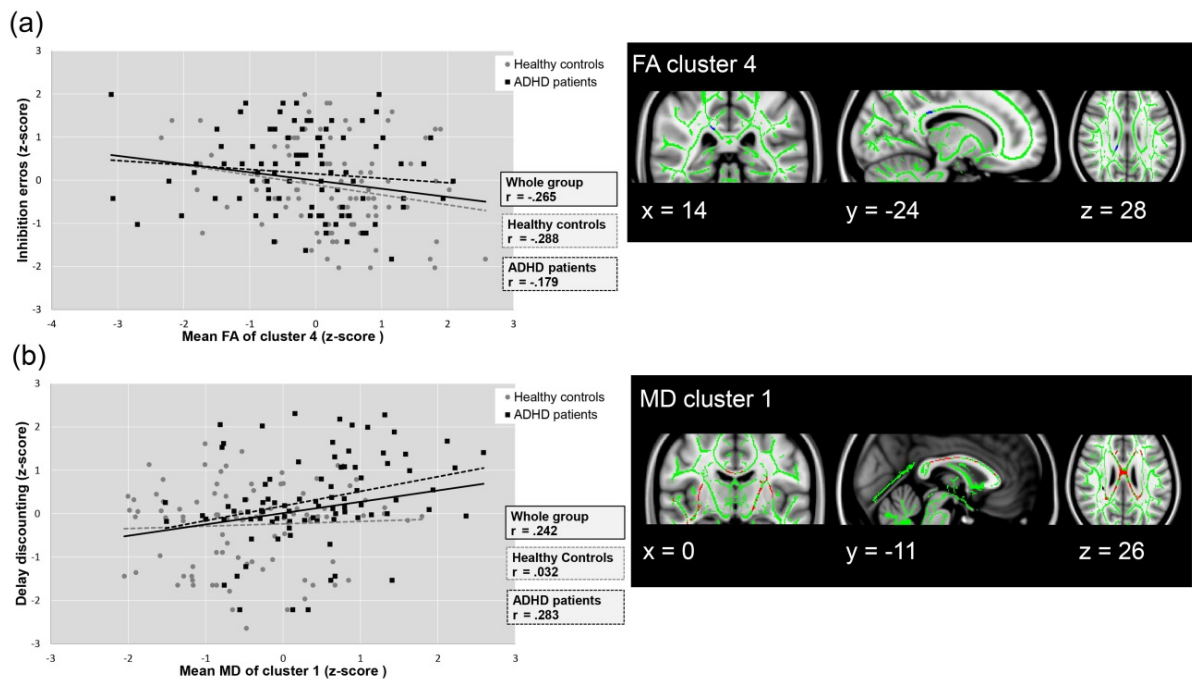


Figure 2. Correlations between inhibition performance and mean FA in cluster 4 (a) and delay discounting score and mean MD in cluster 1 (b) for ADHD patients and controls separately; $*p < .05$. Worse inhibition was reflected by a higher number of commission errors as measured with the Sustained Attention to Response Task (SART) task, and higher impulsivity was reflected by steeper discounting in the Delay Discounting task.

Effect of medication use and depression history on significant between-subject clusters of FA, MD, and RD

Sensitivity analyses were conducted to examine possible effects of stimulant medication use or depression history on significant clusters from the between-group analysis for FA, MD, and RD. Extracted mean values from the significant between-group clusters for FA, MD, and RD did not differ between medication-naïve and stimulant-treated patients ($p > .05$) (Supplementary Table S2). There were no differences on the extracted mean values between ADHD patients with no history of depressive episodes and ADHD patients with one or more episodes in the past ($p > .05$) (Supplementary Table S3).

Discussion

In this study we examined white matter microstructure in adult patients with ADHD and healthy controls. Compared to the healthy individuals, patients with ADHD showed significantly reduced FA and increased MD and RD in several brain regions, but no differences in AD. While FA and MD differences were not related with symptom severity, lower FA in the splenium of the corpus callosum was associated with worse inhibition performance, and higher MD in several ROIs was associated with higher impulsivity.

Strongest effects were found in the body and splenium of the corpus callosum. This supports earlier reports that white matter anomalies in the corpus callosum are one of the most consistently found features in childhood ADHD (Cao et al., 2010; Pavuluri et al., 2009; Qiu et al., 2011; van Ewijk et al., 2014) and adult ADHD (Dramsahl et al., 2012; Konrad et al., 2010), although some studies did not find corpus callosum abnormalities (de Zeeuw et

al., 2012; Hamilton et al., 2008; Hong et al., 2014; Nagel et al., 2011; van Ewijk et al., 2012). Importantly, reduced FA values in the splenium were associated with worse inhibition performance. Poorer response inhibition in healthy children has been correlated previously with decreased FA (and increased MD) in the splenium (Paolozza et al., 2014). It has been linked to decreased splenium volume in children prenatally exposed to polychlorinated biphenyls (Stewart et al., 2003) and in adults with bipolar disorder (Bearden et al., 2011), populations also characterized by insufficient inhibitory control. The splenium of the corpus callosum connects interhemispheric somatosensory, auditory, occipital, and motor areas, which are important for visual object recognition and discrimination. Possibly, commission errors arise due to insufficient transmission of visual information to the brain areas executing inhibitory control. Our results show that the association between splenium FA and inhibition performance was weaker in patients than in healthy individuals, suggesting that this structure is less functional in ADHD patients.

Besides the corpus callosum, the observed differences in posterior and superior regions of the corona radiata are consistent with ADHD studies in childhood (Nagel et al., 2011; Qiu et al., 2011) and adulthood (Cortese et al., 2013). These regions are continuations of the posterior limb of the internal capsule to the sensorimotor cortex and contain axons primarily involved in low-level motor function. Alterations in these tracts might contribute to sensorimotor deficits in adult ADHD (Valera et al., 2010). Compared to controls, ADHD patients showed reduced FA in the posterior thalamic radiation consistent with an earlier finding in adult ADHD (Cortese et al., 2013), although a childhood study showed increased rather than decreased FA in this area (Peterson et al., 2011). The thalamic radiation contains fibers that run towards the occipital cortex carrying visual information and might be related to structural visual cortex abnormalities (Ahrendts et al., 2011) and functional visual deficits (Kim et al., 2014) in adult ADHD.

Our findings of increased MD suggests that white matter cellular density is lower in ADHD patients (Alexander et al., 2007). In agreement with earlier studies (de Luis-Garcia et al., 2015; Lawrence et al., 2013), these findings for MD were observed in more widespread areas of the brain than those for FA and our finds support a recent study that increased MD was correlated with worse performance indicators of ADHD (Conners Continuous Performance Test) (de Luis-Garcia et al., 2015).

Moreover, increased MD within a large cluster encompassing wide-spread regions was associated with steeper delay discounting. Steeper delay discounting occurs when smaller immediate rewards are preferred over larger delayed rewards, and is linked to impulsivity. Earlier studies found similarly that steeper delay discounting was associated with higher MD (and lower FA) in bilateral frontal/temporal lobes and in fronto-striatal tracts (Olson et al., 2009; Peper et al., 2013). A recent resting-state functional connectivity study in childhood ADHD showed that steeper delay discounting was related to differences in reward circuit connectivity (Costa Dias et al., 2013). In conclusion, aberrant structural and functional connectivity possibly influences the balanced interaction between the reward network and other cognitive control regions. This may unveil vulnerability to impulsive decision making in

ADHD. Future research could benefit from using a connectomics approach, combined with multimodal imaging that includes diffusion measures as well as functional MRI (Hong et al., 2014; Shenton et al., 2014).

Decreased FA found in ADHD patients was driven by increases in RD rather than changes in AD. Although the biological correlates of those measures are not yet entirely clarified, it is believed that increases in RD (with minimal changes in AD) reflect decreased myelination, while decreases in AD reflect axonal damage or degeneration (Alexander et al., 2007; Song et al., 2002). Whereas studies in young children and adolescents with ADHD suggest delayed myelination (Nagel et al., 2011; Tamm et al., 2012), our results support the only other adult ADHD study that has investigated AD/RD and point to atypical myelination not only being delayed but rather representing a persistent anomaly in ADHD (Shaw et al., 2015). This implicates myelination as a novel target for genomic studies and for more tailored pharmacological treatment interventions.

We used two approaches to investigate effects of FA and MD on symptom severity. The first approach was a voxel-based regression with TBSS adapted from van Ewijk et al. (2014). The second approach was a conventional ROI analysis using the mean FA and MD of significant between-group clusters as predictors for symptoms. Both approaches yielded non-significant results, consistent with another adult ADHD study showing no association between corpus callosum differences and symptom severity (Dramsdaahl et al., 2012). While this suggests that white matter differences in adult ADHD are independent of disease severity, a vast amount of literature does show relations with severity (Ashtari et al., 2005; Nagel et al., 2011; Shang et al., 2013; Shaw et al., 2015; van Ewijk et al., 2014). A firm conclusion on whether this can be explained by differences based on e.g. the age of the sample will have to await future analyses in larger samples. International collaboration in consortia like the Enabling Neuro Imaging Genetics through Meta-Analysis (ENIGMA) Consortium (www.ENIGMA.ini.usc.edu), which runs a ADHD working group, might provide increased power to clarify this point.

Our findings did not differ between drug-naïve ADHD patients and stimulant medicated patients which supports prior studies that found no confounding effects of (stimulant) medication (de Zeeuw et al., 2012; Hamilton et al., 2008; van Ewijk et al., 2014). Additionally, our findings did not differ between ADHD patients with a history of major depression and ADHD patients without this comorbidity. Since deviant white matter integrity has also been found in numerous psychiatric disorders, it would be of particular interest to go across diagnostic boundaries in future studies and investigate whether certain white matter abnormalities are specific for ADHD or are shared between disorders.

While our DTI study sample is the largest one published to date for adult ADHD, it also has a limitation. We used two different diffusion scan acquisition protocols. However, this could not have biased our results, as group representation did not differ across protocols, and all analyses were performed with protocol as a covariate. Moreover, the same pattern of results held up when the main between-subject TBSS analysis for FA was limited to the single protocol on which most scans were performed, albeit with lower

significance ($P_{FWE} = .10$) (see Supplementary Table 4). Additionally, we could not extensively study the role of comorbid substance abuse, which is an important concern considering the increased risk of substance use disorders in patients with ADHD (Gorzkowska et al., 2014; Wilens, 2004). Adolescent substance use has harmful effects on the development of white matter characteristics (Bava et al., 2013) and prefrontal cortex volume (Lejuez et al., 2010). Importantly, microstructural damage in corpus callosum has been suggested as a risk factor for alcohol use disorders (De Bellis et al., 2008).

In conclusion, this study demonstrates white matter microstructure alterations in adult ADHD and point to abnormal myelination. These white matter changes might represent a core trait of persistent ADHD that is independent of disease severity. The white matter microstructure alterations may have specific functional relevance given that lower FA in the corpus callosum was related to inhibition problems and increased MD in wide-spread tracts was related to impulsive decision making.

Supplementary information

Supplementary Table 1. Tasks and outcome measures of the neurocognitive measures

Task (Cognitive domain)	Description	Outcome measure
1. WAIS-III Digit Span task (Wechsler, 1997) (Working memory)	Digit-strings are read aloud by the experimenter. In the forward condition, the participant is asked to repeat digits in the same order. In the backward condition, the participant is asked to repeat the digits in the reverse order.	Forward digit span score Backward digit span score.
2. Sustained Attention Dots (SA-dots) task (De Sonneville, 1999) (Attention)	Participants are asked to react to a series of dots on the screen; there can be either 3, 4 or 5 dots presented simultaneously. Dots appear in a random order in a paced tempo. When 3 or 5 dots appear on the screen, the participant has to react with the 'no-key' (the key handled by the non-dominant hand) and when 4 dots appear the participant is asked to react with the 'yes-key' (the key handled by the dominant hand). Pressing the 'no-key' when 4 dots appear is called a false alarm. Pressing the 'yes-key' when 3 or 5 dots appear is called a miss. For analysis, the task is split up into 10 blocks, or series, in order to compute variance in performance over time.	Response bias (the difference between the number of misses and the number of false alarms across the entire task).
3. Sustained Attention to Response Task (SART) (Smit et al., 2004) (Inhibition)	Adaptation of the Go/NoGo task. A stream of digits (ranging from 1 to 9) is presented on the screen. The participant is asked to react on these by pressing a button (on a buttonbox). The stimuli ensure that reactions follow a certain pace. To one of the digits, 3, the participant had to withhold a response. A commission error is made when the participant presses the button when a 3 is presented. An omission error is made when the participant does not press the button when a digit that is not 3 is presented.	Number of commission errors
4. Delay Discounting task (Dom et al., 2006) (Delay aversion & impulsivity)	The participant is repeatedly asked to make a choice between two (hypothetical) incentives. One option generates an incentive (money) at a short period while the other option generates an incentive at a later time (i.e. "Do you prefer to receive 30 Euros 180 days from now, or 2 Euros immediately?"). During the task, the value of the incentives as well as the time of the delay (with which the incentive is gained) are varied. The impulsivity parameter (k) is computed from the present value of the delayed reward (V), the real value of the delayed reward (a) and the delay in days (D) with the formula: $V = a / (1+kD)$.	K 100

Supplementary Table 2. Means \pm standard errors of extracted FA, MD, and RD across significant between-group clusters for healthy controls (HC), drug-naïve and stimulant-using patients with ADHD^a

Mean value across significant clusters	HC (N = 109)	ADHD naïve (N = 20)	ADHD stimulants (N = 64)	Significant differences
FA	0.637 \pm 0.002	0.615 \pm 0.005	0.621 \pm 0.003	HC > ADHD naïve & ADHD stimulants; ADHD naïve = ADHD stimulants
MD ^b	0.762 \pm 0.021	0.799 \pm 0.050	0.777 \pm 0.028	HC < ADHD naïve & ADHD stimulants; ADHD naïve = ADHD stimulants
RD ^b	0.495 \pm 0.021	0.522 \pm 0.048	0.506 \pm 0.027	HC < ADHD naïve & ADHD stimulants; ADHD naïve = ADHD stimulants

FA fractional anisotropy, MD mean diffusivity, RD radial anisotropy.

^a Based on the estimated marginal means and controlled for age, gender and DTI acquisition protocol.

^b 10⁻³ mm²/s.

Supplementary Table 3. Means \pm standard errors of extracted FA, MD, and RD across significant between-group clusters for healthy controls (HC), ADHD patients with no history of depressive episodes and ADHD patients with one or more episodes in the past ADHD^a

Mean value across significant clusters	HC with no history of depressive episodes (N = 95)	ADHD patients with no history of depressive episodes (N = 43)	ADHD patients with one or more episodes in the past (N = 64)	Significant differences
FA	0.636 \pm 0.002	0.619 \pm 0.003	0.619 \pm 0.003	HC > ADHD no depr & ADHD depr; ADHD no depr = ADHD depr
MD ^b	0.761 \pm 0.020	0.783 \pm 0.030	0.781 \pm 0.030	HC < ADHD no depr & ADHD depr; ADHD no depr = ADHD depr
RD ^b	0.494 \pm 0.020	0.512 \pm 0.030	0.511 \pm 0.030	HC < ADHD no depr & ADHD depr; ADHD no depr = ADHD depr

FA fractional anisotropy, MD mean diffusivity, RD radial anisotropy.

^a Based on the estimated marginal means and controlled for age, gender and DTI acquisition protocol.

^b 10⁻³ mm²/s.

Supplementary Table 4. Clusters showing significant differences in Fractional Anisotropy (FA) between ADHD (N=72) patients and healthy controls (N=86) with the newest DTI protocol

Clusters with significantly lower FA in ADHD patients					
Cluster	White matter tracts overlapping with the clusters (size of overlap in >10 voxels) ^a	Size (voxels) ^b	MNI coordinates (x;y;z)	Partial eta ²	p ^d
1	Body and splenium of CC, Posterior limb of IC (R), Retrolenticular part of IC (R), SCR R (R), PCR (R), PTR (R), EC (R), SLF (R), Tapetum (R)	3912	16;-18;33	.174	.056
2	PTR (R)	365	26;-74;14	.106	.089
3	Body of CC, SCR (R)	151	17;8;33	.060	.091
4	Unclassified	123	40;-27;52	.131	.091
5	Genu and body of CC, ACR (R)	116	12;22;19	.052	.098
6	Unclassified	71	13;-79;23	.103	.091
7	Unclassified	56	28;-82;2	.066	.098
8	PCR (R), PTR (R)	25	31;-63;18	.042	.099

CC corpus callosum, ACR anterior corona radiata, FA fractional anisotropy, MD mean diffusivity, PCR posterior corona radiata, RD radial anisotropy, SCR superior corona radiata, RPIC retrolenticular part of IC, PTR posterior thalamic radiation (include optic radiation), PLIC posterior limb of IC, SLF superior longitudinal fasciculus, IC internal capsule, EC external capsule, SFOF superior fronto-occipital fasciculus, UF uncinat fasciculus.

^a White matter tracts as defined with the Johns Hopkins University White Matter Label Atlas.

^b Cluster size > 10 voxels.

^c Partial eta squared based on mean FA, MD and RD of the cluster.

^d $p < .05$, FWE-corrected, controlling for gender, age and scan acquisition protocol.

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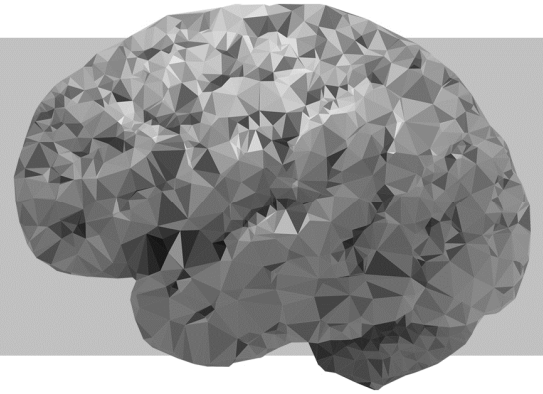
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Lower white matter microstructure in the superior longitudinal fasciculus is associated with increased response time variability in adult with ADHD

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Abstract

Response time variability (RTV) is consistently increased in patients with attention-deficit/hyperactivity disorder (ADHD). A right-hemispheric frontoparietal attention network model has been implicated in these patients. The 3 main connecting fibre tracts in this network, the superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF) and the cingulum bundle (CB), show microstructural abnormalities in patients with ADHD. We hypothesized that the microstructural integrity of the 3 white matter tracts of this network are associated with ADHD and RTV.

Methods: We examined RTV in adults with ADHD by modelling the reaction time distribution as an exponentially modified Gaussian (ex-Gaussian) function with the parameters μ , σ and τ , the latter of which has been attributed to lapses of attention. We assessed adults with ADHD and healthy controls using a sustained attention task. Diffusion tensor imaging-derived fractional anisotropy (FA) values were determined to quantify bilateral microstructural integrity of the tracts of interest.

Results: We included 100 adults with ADHD and 96 controls in our study. Increased τ was associated with ADHD diagnosis and was linked to symptoms of inattention. An inverse correlation of τ with mean FA was seen in the right SLF of patients with ADHD, but no direct association between the mean FA of the 6 regions of interest with ADHD could be observed.

Limitations: Regions of interest were defined a priori based on the attentional network model for ADHD and thus we might have missed effects in other networks.

Conclusion: This study suggests that reduced microstructural integrity of the right SLF is associated with elevated τ in patients with ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is characterized by a pervasive pattern of age-inappropriate inattentive behaviour and/or impulsiveness and hyperactivity. Although ADHD is often viewed as a childhood disorder, it also affects 2.5% of adults (Simon et al., 2009) which has profound negative implications for the patients themselves, their social environment and society. The pathophysiology of ADHD is still poorly understood owing in part to its substantial clinical and etiological heterogeneity (Biederman, 2005).

Increased response time variability (RTV) is one of the most common characteristics of patients with ADHD. Response time variability is the moment-to-moment fluctuation of performance in neuropsychological response time experiments and has been found to be increased in patients with ADHD across a number of different experimental paradigms, including sustained attention, flanker interference and working memory tasks (for reviews see the studies by Tamm and colleagues (2012) and Kofler and colleagues (2013)). Recent studies suggest that increased performance variability in patients with ADHD might be a neurocognitive marker and may serve as a potential endophenotype for this disorder (Frazier-Wood et al., 2012; Kuntsi and Klein, 2012). In addition to capturing RTV through standard deviation (SD) of response time (RT), other quantitative measures of RTV have also been reported. The exponentially modified Gaussian (ex-Gaussian) method, for example, provides quantification of the shapes of RT frequency distributions in the form of 3 different parameters. Two parameters reflect the central tendency (μ) and variance (σ) of the Gaussian component. An exponential component, τ , provides information on the extent of the positive skew of RT distributions. Increased τ indicates more frequent excessively long RTs and is often attributed to lapses in attention (Epstein et al., 2011; Gu et al., 2013; Hervey et al., 2006; Leth-Steensen et al., 2000). Increased τ has consistently been found in patients with ADHD across tasks (Buzy et al., 2009; Epstein et al., 2011; Geurts et al., 2008; Gu et al., 2013; Hervey et al., 2006; Kuntsi and Klein, 2012; Leth-Steensen et al., 2000; Lin et al., 2013; Vaurio et al., 2009). Compared with reports on τ , reports on alterations of μ and σ in patients with ADHD have been less consistent, and findings seem more task-dependent (Epstein et al., 2011). The disorder has been associated with decreased μ (Buzy et al., 2009; Gu et al., 2013; Hervey et al., 2006), increased σ (Buzy et al., 2009; Gu et al., 2013; Hervey et al., 2006; Lin et al., 2013; Vaurio et al., 2009), or no differences in μ (Geurts et al., 2008; Leth-Steensen et al., 2000; Lin et al., 2013; Vaurio et al., 2009) or σ (Geurts et al., 2008; Leth-Steensen et al., 2000). These studies show that ADHD-related RTV is predominantly determined by extremely long RTs, which are best captured by an elevation in τ . Studies disentangling the neurobiological substrates of τ are limited but may lead to a better understanding of attention processes in RT paradigms, especially in ADHD.

Russel and colleagues (2006) hypothesized that reduced myelination of white matter might be a neurobiological mechanism responsible for increased performance variability in patients with ADHD. Recently, RTV has been associated with intersubject variability in the microstructure of different white matter tracts. Using diffusion tensor imaging (DTI) and deriving fractional anisotropy (FA) coefficients reflecting the microstructural integrity of

white matter (Basser, 1995), significant correlations were established between performance variability measured across different simple RT tasks and white matter microstructure (Fjell et al., 2011; Moy et al., 2011). During the course of normal human postnatal development, RTV is known to change in a U-form fashion, with a decrease in variability throughout childhood and a later increase in adulthood (Williams et al., 2005). The reduction of RTV during adolescence has been partially linked to the maturation of white matter tracts in the brain (Tamnes et al., 2012). A recent study performed in young patients with ADHD and healthy controls showed that FA of the left cingulum bundle (CB) was negatively correlated with τ only in patients with ADHD (Lin et al., 2013). Importantly, the CB is part of the attentional network model as defined by Makris and colleagues (2009). These researchers defined several neuroanatomical models of distributed network dysfunction that may lead to symptoms of ADHD. Brain regions in the attentional network are the right hemispheric frontoparietal cortices, the thalamus and the cerebellum. Besides the CB, two main fibre pathways connecting these regions are the superior longitudinal fasciculus (SLF) and the inferior longitudinal fasciculus (ILF). The SLF is a bidirectional link between regions in frontal and parietal cortices (Makris et al., 2009; Makris et al., 2005). White matter microstructure of the SLF has been shown to be compromised in children (Lawrence et al., 2013; van Ewijk et al., 2014) and in adults with ADHD (Hamilton et al., 2008), especially in the right-lateralized part (Cortese et al., 2013; Makris et al., 2008). Another study showed that task-related measures of attentional performance in adults with ADHD correlated significantly with FA in the right SLF (Konrad et al., 2010). The ILF connects the occipital with the temporal cortices (Makris et al., 2009), and bilateral regions of the ILF are also abnormal in children and adults with ADHD (Cortese et al., 2013; Silk et al., 2009). Furthermore, the microstructural integrity of the right CB was found to be altered in adults with ADHD (Makris et al., 2008). Altogether, these findings suggest that the microstructural integrity of these fibre tracts, especially in the right hemisphere, might contribute to the complex etiology of attentional problems in patients with ADHD and subsequently may be partly responsible for a greater prevalence of extremely long RT in patients with ADHD, as reflected in the higher ex-Gaussian component τ in this group.

Here, we aimed to advance existing knowledge on the association between ADHD, τ and microstructural integrity of the SLF, ILF and CB. Based on the white matter tracts described in the attention network model by Makris and colleagues (2009), we used a hypothesis-driven region of interest (ROI) approach in a large sample of well-characterized adult patients with ADHD and healthy controls. We hypothesized that patients with ADHD would show elevated τ , especially linked to higher rates of inattention symptoms. Furthermore, we expected that ADHD and τ would be linked to reduced mean FA in the SLF, ILF and/or CB white matter tracts, with higher values on τ reflecting attention lapses associated with lower mean FA most prominently in right-lateralized white matter tracts.

Methods

Participants

We recruited individuals from the Dutch cohort of the International Multicentre persistent ADHD CollaboraTion (IMpACT; www.impactADHDgenomics.com) (Franke et al., 2010) to participate in our study. The patients with ADHD were recruited from the Department of Psychiatry, Radboud University, Nijmegen, the Netherlands, and healthy controls were recruited through advertisements.

All participants underwent psychiatric assessments, neurocognitive tests and neuroimaging. Participants were assessed using the diagnostic interview for adult ADHD (DIVA) (Kooij, 2012). This interview focuses on the 18 DSM-IV symptoms of ADHD and uses concrete and realistic examples to investigate whether a symptom is currently present or whether it was present in childhood. We acquired supplementary information from parents and school reports whenever possible. In addition, we administered the ADHD Rating Scale-IV, a self-report questionnaire on current symptoms of attention and hyperactivity/impulsivity (Kooij et al., 2005). Patients were included in the study if they met DSM-IV-TR criteria for ADHD in childhood as well as in adulthood. We used the Structured Clinical Interviews for DSM-IV (SCID-I and SCID-II) (Groenestijn et al., 1999; Weertman et al., 2000) for comorbidity assessment. The assessments were carried out by trained professionals (i.e., psychiatrists or psychologists). Exclusion criteria were psychosis, alcohol or substance addiction in the 6 months preceding the study, current major depression, full-scale IQ estimate lower than 70 (prorated from the Block Design and Vocabulary components of the Wechsler Adult Intelligence Scale-III), neurologic disorders, sensorimotor disabilities, non-Caucasian race and use of medications other than psychostimulants or atomoxetine. Healthy controls who had a current neurologic or psychiatric disorder according to DIVA, SCID-I or SCID-II or who had any first-degree relatives with ADHD or another major psychiatric disorder were excluded. Because our study is part of a genetics project, all participants were Dutch and of European Caucasian ancestry to reduce potential genetic heterogeneity. The regional ethics committee (Centrale Commissie Mensgebonden Onderzoek: CMO Regio Arnhem — Nijmegen; protocol number III.04.0403) approved our study, and we obtained written informed consent from all participants.

Characterization of the RT frequency distribution in a sustained attention task

The ex-Gaussian parameters (μ , σ and τ) were derived from a sustained attention (SA)-dots task (De Sonneville, 1999) in which a pattern of 3, 4 or 5 dots was presented on a computer screen in a random order. Participants had to indicate as quickly and accurately as possible how many dots were present by pressing a button with their dominant (4 dots) or nondominant hands (3 and 5 dots). Owing to the duration (approximately 12 min consisting of 600 trials in total with 200 trials per condition) and complexity, this task forms a good basis for quantification of RTV with the ex-Gaussian method. All premature responses ($RT < 150$ ms) were excluded before the determination of the 3 ex-Gaussian parameters (Geurts et al., 2008). In addition, we excluded error trials and the trial subsequent to errors, as these

could reflect posterror slowing (Epstein et al., 2010). An ex-Gaussian probability density function was then fit to the RT histogram for each participant. The optimal values for the 3 parameters (μ , σ and τ) of the ex-Gaussian function were determined with the Simplex search method described by Lacouture and Cousineau (2008) using MATLAB (Mathworks Inc.).

DTI acquisition

Whole brain imaging was performed with a 1.5 T MR scanner (Magnetom Avanto, Siemens Medical Systems) and a standard 8-channel head coil. We obtained a high-resolution T1-weighted magnetization-prepared rapid gradient-echo anatomic scan from each participant in which the inversion time (TI) was chosen to provide optimal grey matter–white matter T1 contrast (repetition time [TR] 2730 ms, echo time [TE] 2.95 ms, TI 1000 ms, flip angle 7°, field of view [FOV] 256 × 256 × 176 mm³, voxel size 1.0 × 1.0 × 1.0 mm³). The T1 images served as high-resolution reference images for diffusion imaging data. Transversely oriented diffusion-weighted images were acquired using a twice-refocused spin-echo-planar-imaging sequence that minimized imaging distortions from eddy currents (Reese et al., 2003). The diffusion imaging data were acquired using 2 different protocols. Sixty participants were scanned with the following protocol: TR 10 200 ms, TE 95 ms, FOV 320 × 320 × 160 mm³, voxel size 2.5 × 2.5 × 2.5 mm³, 6/8 partial Fourier. Four images without diffusion weighting ($b = 0$ s/mm²) and 30 images with diffusion weighting ($b = 900$ s/mm², 34 diffusion directions) applied along noncollinear directions were acquired. The remaining 137 participants were scanned with an adapted second protocol that was implemented to reduce motion artifacts during scanning. Parameters that differed from the first protocol were TR (6700 ms), TE (85 ms) and FOV (220 × 220 × 140 mm³), and scans were acquired with full Fourier acquisition; other parameters were unchanged. For each slice, the diffusion weighting for the 30 images changed to $b = 900$ s/mm². We corrected for possible variance introduced owing to the different protocols by including the diffusion-weighted acquisition protocol as a confound in the analyses.

Image preprocessing

The diffusion-weighted data were preprocessed using an algorithm developed in house. In short, the diffusion-weighted images of each participant were realigned on the unweighted image using mutual information routines from SPM5. Next, we used an iteratively reweighted-least squares algorithm to correct for head and cardiac motion artifacts in the diffusion-weighted data (Zwiers, 2010). Using DTIFIT within the FMRIB Diffusion Toolbox (part of FMRIB's Software Library [FSL]), FA images were created and subsequently fed into the tract-based spatial statistics (TBSS) pipeline (Smith et al., 2006). Here, all individual FA maps were nonlinearly registered to the FMRIB58_FA template using FSL's nonlinear registration tool FNIRT and then affine-transformed into standard Montreal Neurological Institute (MNI) space. A mean FA image was created and thinned to create a mean FA skeleton, which represents the centres of all tracts common to the group. A threshold of 0.2

was used to avoid partial voluming effects. Individual FA skeleton images were then mapped onto this mean skeleton for statistical evaluation.

We used the Johns Hopkins University white matter tractography atlas (Hua et al., 2008) with a probability threshold of 0.5 to identify regions on the mean FA skeleton corresponding to the left and right SLF and ILF. The ICBM-DTI-81 white matter labels atlas (Mori et al., 2008) was used to identify regions on the mean FA skeleton corresponding to the left and right CB. For optimal accuracy, both atlases were nonlinearly registered to the FMRIB58 brain. To obtain mean FA values for each ROI for each participant, each mask was multiplied with the mapped individual's FA skeleton images and averaged (Figure 1).

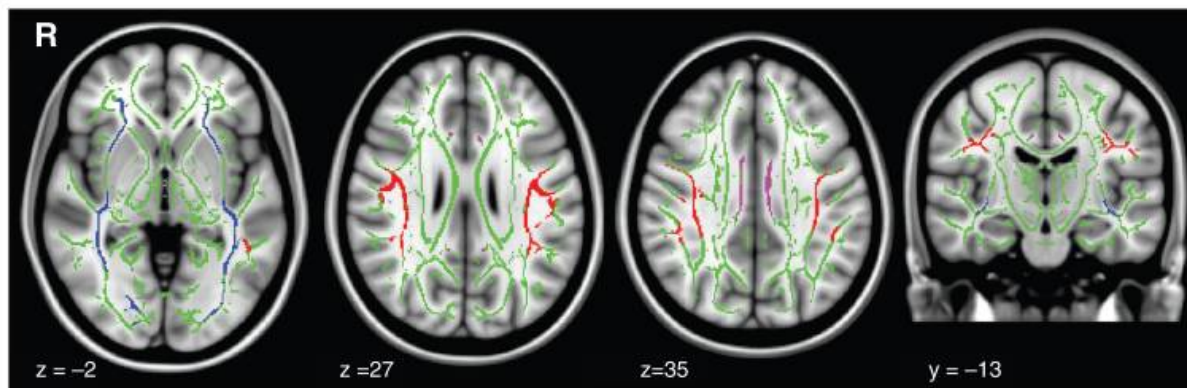


Figure 1. Aligned and binarized masks of skeletonized white matter tracts for (blue) the left (1975 voxels) and right (2198 voxels) inferior longitudinal fasciculus, (red) the left (2780 voxels) and right (2248 voxels) superior longitudinal fasciculus, and (purple) the left (416 voxels) and right (401 voxels) cingulum bundle. The mean fractional anisotropy skeleton mask is shown in green.

Statistical Analyses

The distributions of the 3 ex-Gaussian parameters were normalized by taking their natural logarithm. Outliers were defined as residuals with a larger or smaller value than 3 times the SD of the predicted value. One patient with ADHD had an extreme measurement of τ and was therefore excluded from all further analyses. We used an analysis of covariance (ANCOVA) model to assess whether the ex-Gaussian parameters (μ , σ and τ) were significantly different between groups, adjusting for age, sex and handedness. Then, separately for both groups, we performed ordinal regression analyses to examine the association of τ with self-reported symptoms of inattention and hyperactivity/impulsivity. These ordinal regressions were performed controlling for age, sex and handedness. To inspect the individual effects of each ROI on τ , we obtained standardized residuals adjusted for age, sex, handedness, DTI protocol and mean FA of the other ROIs. Those standardized and corrected residuals for each ROI were associated with τ in separate linear regressions. We then performed the same analyses separately for patients with ADHD and controls to address the specificity of the results for each group. Finally, logistic regression analyses were performed using the standardized residuals to inspect whether the mean FA of the 6 ROIs were associated with ADHD diagnosis. We applied Bonferroni correction to adjust for multiple comparisons. Given that we performed 15 independent statistical tests (i.e., 3 analyses including the ex-Gaussian parameters, 6 for the association between the ROIs and τ

and 6 for the association between the ROIs and ADHD), we considered results to be significant at $p < 0.0033$.

Results

Demographic and clinical characteristics

In total, we recruited 197 participants (101 adults with ADHD and 96 healthy controls); we excluded 1 outlier, yielding a final sample of 100 adults with ADHD and 96 controls. Demographic characteristics of the study sample are presented in Table 1. There were no significant differences between patients with ADHD and controls with respect to age ($p = 0.58$, $d = 0.03$) or estimated IQ ($p = 0.58$, $d = 0.10$). Patients with ADHD and controls showed the expected differences in self-reported inattention ($p < 0.001$, $d = -3.631$) and hyperactive/impulsive symptoms ($p < 0.001$, $d = -2.647$). Groups were equally distributed with respect to sex ($\chi^2 = 0.450$, $p = 0.56$), handedness ($\chi^2 = 0.925$, $p = 0.40$) and DTI acquisition protocols ($\chi^2 = 3.922$, $p = 0.06$). There were more women than men in our sample (63 women and 38 men in the ADHD group, and 55 women and 41 men in the control group).

Table 1. Demographic and clinical characteristics of the study sample

Characteristic	Group; mean \pm SD or N	
	ADHD, N = 101	Control, N = 96
Male:Female	38:63	41:55
Age (years)	35.83 \pm 11.16	36.17 \pm 11.17
IQ estimate ^a	108.08 \pm 14.92	109.68 \pm 15.34
Inattentive symptoms ^b	6.38 \pm 2.04*	0.55 \pm 0.99*
Hyperactive/impulsive symptoms ^b	5.56 \pm 2.24*	0.79 \pm 1.21*
Number of Errors	27.8 \pm 20.08*	16.94 \pm 11.26*
Medication naïve	16	-
Current medicated		-
Amphetamine	10	
Methylphenidate	56	
Non-stimulant	3	
Past treatment	16	-
One or more depressive episode (remitted) ^c	44	9
Anxiety disorder (remitted) ^c	23	5
Substance abuse (remitted) ^c	18	7
Borderline ^c	9	0
Antisocial ^c	3	0
Right handed	85	86
DTI acquisition protocol 1 ^d	64	73

ADHD = attention-deficit/hyperactivity disorder; DTI = diffusion tensor imaging; SD = standard deviation.

*Indicates a significant difference between ADHD patients and Comparison subjects

^a Scores represent the average of the standard scores for the block design and vocabulary assessments of the Wechsler Adult Intelligence Scale-III.

^b As measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005)

^c As measured by the Structured Clinical Interview for DSM-IV for axis I (Groenestijn et al., 1999) and axis II (Weertman et al., 2000) disorders.

^d First version of DTI acquisition protocol.

Case-control analysis of ex-Gaussian parameters (μ , σ , and τ) and association with self-reported symptoms

The ex-Gaussian parameter μ was significantly lower in patients with ADHD than in controls ($F_{1,191} = 6.73$, $p = 0.010$). No differences were found for values of σ ($F_{1,191} = 3.71$, $p = 0.06$). The strongest effect was for τ , which was significantly increased in patients with ADHD compared with controls ($F_{1, 191} = 24.96$, $p < 0.001$; Table 2). After correcting for multiple testing, only τ remained significant. The probability density function was more positively skewed in patients than in controls (Figure 2). The estimated mean log-likelihood values for the goodness of fit of the ex-Gaussian probability density function in patients and controls were $3.586e+03 \pm 0.253e+03$ and $3.594e+03 \pm 0.201e+03$, respectively. This difference was not significant (t_{194} , $p = 0.80$). Ordinal regressions showed that in patients with ADHD the parameter τ was significantly associated with self-reported symptoms of inattention (Nagelkerke $R^2 = 0.097$, $p = 0.002$; Supplementary Table 1), and this association was not observed in controls ($p = 0.32$; Supplementary Table 1). Hyperactive/impulsive symptoms were not associated with τ in the ADHD group ($p = 0.51$) or in the control group ($p = 0.97$; Supplementary Table 1). Correction for IQ did not change the results.

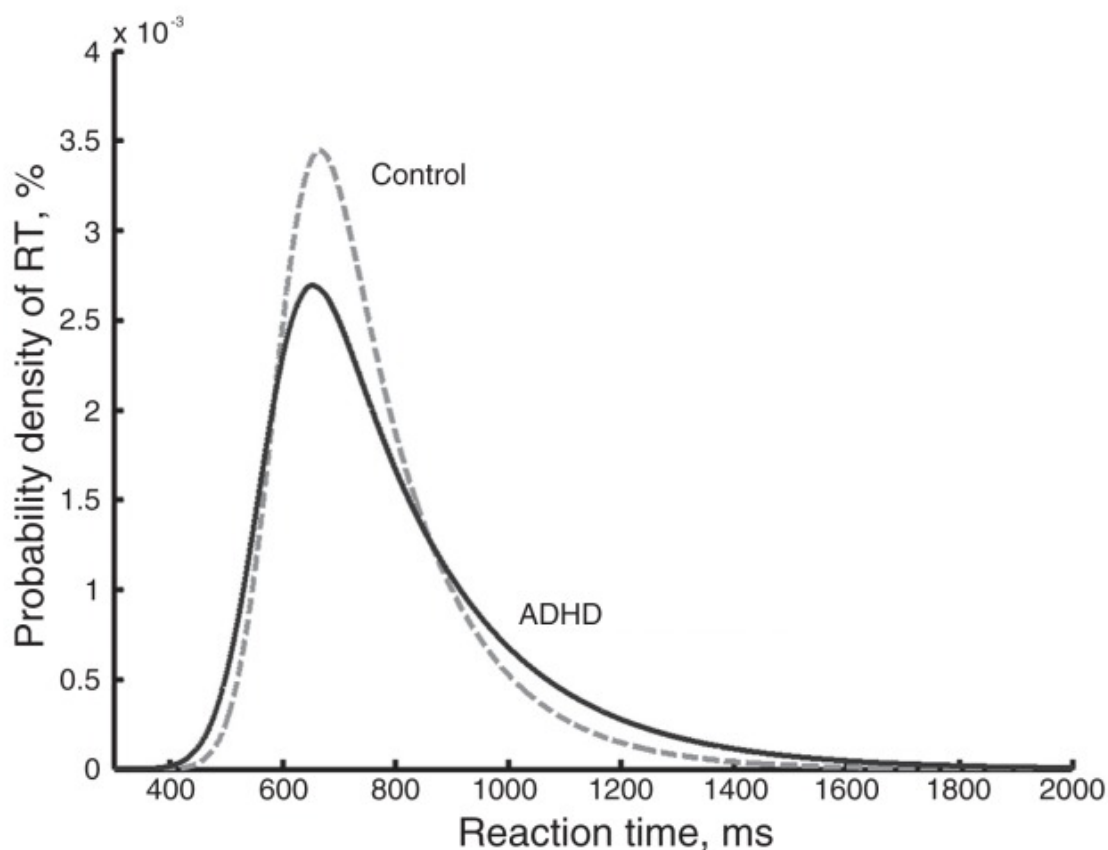


Figure 2. The exponentially modified Gaussian probability density function (PDF) across patients with ADHD and controls. Patients with ADHD have a more positive skewed PDF than controls. RT = response time.

Table 2. ANCOVA analyses of exponentially modified Gaussian parameters for differences between patients with ADHD and controls

Variable	Group, mean \pm SD		Statistic ^a	<i>p</i> -value	Effect size ^a
	ADHD (N = 100)	Control (N = 96)			
Mu (μ)	568.70 \pm 79.67	596.70 \pm 75.10	$F_{1,191} = 6.73$.010	0.034
Sigma (σ)	64.04 \pm 18.76	58.85 \pm 16.03	$F_{1,191} = 3.71$.056	0.019
Tau (τ)	215.16 \pm 95.99	158.46 \pm 55.03	$F_{1,191} = 24.96$	< .001	0.116

ADHD = attention-deficit/hyperactivity disorder; SD = standard deviation.

^a Corrected for age, gender and handedness

Association analysis of mean FA of SLF, ILF and CB with τ

The mean FA value of the right SLF was associated with τ ($R^2 = 0.044$, $p = 0.003$), with higher mean FA associated with lower values of τ (Table 3). This finding remained significant after correcting for multiple testing. Sensitivity analyses for each ROI showed that the effects remain stable when not controlling for the other ROIs (Supplementary Table 2). Furthermore, we performed extra sensitivity analyses to inspect whether the effect remained stable if all non-error trials were included to determine the ex-Gaussian parameters (Supplementary Table 3), if all trials were included to determine the ex-Gaussian parameters (Supplementary Table 4) and if controlling for the number of errors in our analyses (Supplementary Table 5). All separate sensitivity analyses were consistent with the effects found in the main analyses. Separate analyses for patients with ADHD and controls showed that the association between the mean FA value of the right SLF and τ was specific to the ADHD group ($R^2 = 0.098$, $p = 0.002$), as there was no association in controls ($p = 0.56$; Figure 3 and Supplementary Table 6). None of the other 5 ROIs was associated with τ (all p 's > 0.05; Table 3), and no direct association was observed between ADHD diagnosis and mean FA in the 6 ROIs (all p 's > 0.05; Supplementary Table 7). Correction for IQ did not change the results. Furthermore, we performed sensitivity analyses to show that the direction of effects across DTI protocols remained the same (Supplementary Table 8).

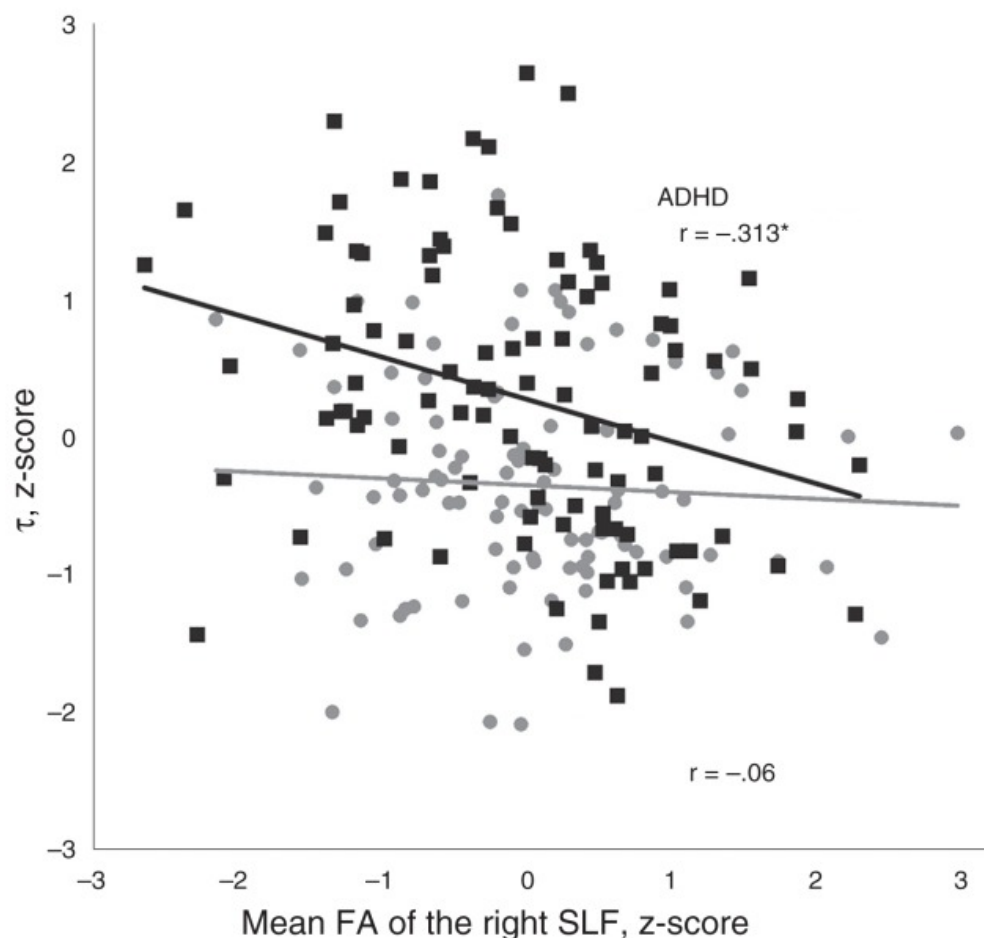


Figure 3. Semi-partial correlation between τ and mean fractional anisotropy (FA) of the right superior longitudinal fasciculus (SLF), separately for patients with ADHD and controls. Both variables were standardized to z-scores. *Significant correlation between FA of the right SLF and τ .

Table 3. Single regression analyses associating Tau (τ) with mean FA values of bilateral ROIs of SLF, ILF and CB corrected for age, gender, handedness and DTI-protocol

FA residuals	Dependent variable: Tau (τ)			p -value	β	R^2
	N	b \pm SE	Statistic			
Left SLF	196	1.282 \pm 2.974	$T_{195} = 0.431$.667	.031	.001
Right SLF	196	-8.675 \pm 2.910	$T_{195} = -2.981$.003	-.209	.044
Left ILF	196	-.813 \pm 2.975	$T_{195} = -0.273$.785	-.020	.001
Right ILF	196	3.053 \pm 2.967	$T_{195} = 1.029$.305	.074	.005
Left CB	196	1.682 \pm 2.973	$T_{195} = 0.566$.572	.041	.002
Right CB	196	0.234 \pm 2.975	$T_{195} = 0.079$.937	.006	.001

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each ROI, the effects of age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

Discussion

In this study we investigated the association of the shape of RT distribution with white matter integrity of a priori ROIs within the attention network model in a large sample of adults with ADHD. We found decreased μ and increased τ in patients with ADHD compared with controls. Consistent with our hypothesis, the strongest effect was found for τ , and increased levels of τ were also associated with increased symptoms of inattention. We found τ to be linked to the microstructural white matter integrity of the right SLF, with increased levels of τ being associated with lower mean FA. This effect was specific to the patients with ADHD.

Several studies in children and adolescents have applied ex-Gaussian analyses to RT data and have shown consistently that increased τ is associated with ADHD (Buzy et al., 2009; Epstein et al., 2011; Geurts et al., 2008; Gu et al., 2013; Hervey et al., 2006; Kuntsi and Klein, 2012; Leth-Steensen et al., 2000; Lin et al., 2013; Vaurio et al., 2009). To our knowledge, the present study is the first of this size performed in adults that used ex-Gaussian analyses to demonstrate that these childhood findings may represent a persistent deficit in patients with ADHD. In addition, the correlation of τ with inattention symptoms supports earlier literature suggesting that excessively long RTs represent lapses of attention (Epstein et al., 2011; Gu et al., 2013; Hervey et al., 2006; Leth-Steensen et al., 2000).

We showed that increased τ is associated with lower FA values in the right SLF. Thus, within the attentional network model (Makris et al., 2009), the white matter microstructure of the SLF might be a particularly important mechanism underlying poor attention in patients with ADHD. We could not replicate an earlier finding by Lin and colleagues (2013), who showed that the CB was associated with μ and τ in patients with ADHD; this could be explained by sample and/or methodological differences between our studies. Lin and colleagues investigated children and adolescents ($N = 56$) and used individual-based tractography, which is different from our atlas-based DTI method. The present study did confirm our expectation that the association between mean FA and τ is lateralized to the right hemisphere. Asymmetry in the brain substrates of attention has been the subject of much research (Makris et al., 2009; Thiebaut de Schotten et al., 2011), and two recent meta-analyses of functional MRI studies have suggested that altered brain activity in a right-hemispheric network underlies attention problems in patients with ADHD (Cortese et al., 2012; Hart et al., 2012). Several studies have pointed out that the right-hemispheric dominance for attentional functions has an anatomic basis (Makris et al., 2008; Shinoura et al., 2009; Thiebaut de Schotten et al., 2011). Children and adults with ADHD show attention difficulties for the left visual field (Carter et al., 1995; Epstein et al., 1997; ter Huurne et al., 2013) and an altered architecture of right-hemispheric attentional mechanisms, and more specifically, deficits in the right anterior region have been implicated (Epstein et al., 1997). Our results extend these findings by pinpointing reduced white matter microstructural integrity in the right SLF as a potential locus for impaired response stability as measured with τ . This finding may thus contribute to a better understanding of lateralized attentional deficits in patients with ADHD.

In contrast to our expectations, we could not replicate a direct association between FA in the 6 ROIs with the categorical diagnosis of ADHD. The absence of a significant association between FA values and diagnosis in our study, despite earlier findings of such a link (Cortese et al., 2013; Hamilton et al., 2008; Makris et al., 2008), might be explained by the heterogeneity of the ADHD phenotype (Biederman, 2005). ADHD is likely to have multiple neurobiological causes (Franke et al., 2011; Makris et al., 2009) and this might lead to difficulties finding a direct association between ADHD and a specific neurobiological marker consistently. Variations at the neural system level might be more closely linked to τ than the category ADHD as such. Thus, reducing phenotypic heterogeneity at the clinical diagnostic level by studying ADHD-linked quantitative traits at the neurocognitive level, which are more biologically based (such as τ), could help to elucidate the neurobiological substrates underlying specific behavioural aspects of ADHD.

Our study might have implications beyond ADHD, as elevated τ has been observed not only in patients (children and adults) with ADHD, but also in patients with autism (Geurts et al., 2008), schizophrenia (Kaiser et al., 2008) and bipolar disorder (Bora et al., 2006). Further research on the microstructural white matter integrity of the SLF might also be interesting in these disorders. Moreover, mean FA of the SLF is considerably heritable (58%)(Kochunov et al., 2010) and may be useful as an endophenotype explaining genetic effects on ADHD and other psychiatric disorders as it is potentially more directly linked to gene expression than a clinical diagnosis (Frazier-Wood et al., 2012; Makris et al., 2009).

Limitations

In addition to the clear strengths of this study, which lie in the size and the deep phenotyping of our adult sample, several limitations must also be taken into account when considering the present findings. First, we used an ROI approach to control for type I error by limiting the number of statistical tests instead of correcting for the large number of voxels in the brain. As our a priori hypothesis entailed that τ reflects attentional processes, we based our ROIs on the attentional network model of Makris and colleagues (2009) and on previous work by Lin and colleagues (2013) and Konrad and colleagues (2010). However, while providing the largest statistical power, our ROI approach did not allow us to formulate novel hypotheses about other regions important for attention and ADHD; whole brain analyses in larger samples will be necessary. Similarly, as we used the diagnostic measure only to link FA values, we might have missed more specific links with quantitative measures of inattention and hyperactivity/impulsivity. A further potential limitation of our study might lie in the SLF consisting of four separate components, the SLF I, SLF II, SLF III and the arcuate fascicle (Makris et al., 2005). Specifically, the SLF II has been associated with FA reductions in adults with ADHD (Makris et al., 2008). We quantified the FA in the entire SLF after skeletonization in order to account for realignment issues. However, with this procedure the subcomponents of the SLF could not be evaluated. Given that the SLF components could have different functional affiliations, we may have missed more regionally localized effects. In future research, it might be interesting to inspect other diffusion parameters, such as

mean, axial or radial diffusivity (Alexander et al., 2007), which we did not investigate in the present study. Furthermore, a combination of diffusion imaging with electrophysiological methodologies might provide more insight into the mechanisms underlying moment by moment fluctuations in performance, which might be associated with the energy metabolism in the brain (Russell et al., 2006). Such an explanation of RT variability would be well in line with the cognitive-energetic model of ADHD (Sergeant, 2000). A combination of these methodologies would allow inspecting electrophysiological correlates of attention over time and their link to the microstructure of the right SLF in patients with ADHD. A final potential limitation of this study is that diffusion imaging data were acquired using 2 different protocols. This might have reduced the power of our analyses, but should not have influenced our results in other ways. First, we performed all analyses controlling for scanner acquisition protocol even though group and sex representation did not differ significantly across scanner acquisition protocols. Second, performing the association analysis of mean FA of the SLF, ILF and CB with τ and ADHD diagnosis for the 2 scan protocols separately confirmed the same pattern of results in both (Supplementary Table 8).

Conclusion

The present results shed more light on the complex association between performance variability (as indexed by more frequent excessively long RTs), attention problems and microstructural white matter abnormalities of the right SLF in adults with ADHD.

Supplementary information

Supplementary Table 1. Ordinal regression analyses associating symptom rates with the exponentially modified Gaussian parameters for patients with ADHD and controls, separately corrected for age, sex and handedness

FA residuals*	Dependent variable: Tau (τ)			T_{195}	p -value	β	R^2
	N	$b \pm SE$					
Left SLF	196	-3.832 ± 2.923		-1.311	.191	-.094	.009
Right SLF	196	-8.446 ± 2.873		-2.940	.004	-.207	.043
Left ILF	196	-1.446 ± 2.934		-.493	.623	-.035	.001
Right ILF	196	0.099 ± 2.936		.034	.973	.002	.001
Left CB	196	0.664 ± 2.936		.226	.821	.016	.001
Right CB	196	-0.374 ± 2.936		-.172	.899	-.009	.001

ADHD = attention-deficit/hyperactivity disorder; SE = standard error.

^aAs measured with the ADHD-DSM-IV self-rating scale (Kooij et al., 2005).

Supplementary Table 2. Single regression analyses associating τ with mean FA values of bilateral ROIs of SLF, ILF and CB corrected for age, sex, handedness and DTI protocol but not for the other ROIs

	ADHD patients (N = 100); dependent variable: Tau (τ)				Control (N = 96); dependent variable: Tau (τ)			
	$b \pm SE$	Wald- χ^2	p -value	Nagelkerke's R^2	$b \pm SE$	Wald- χ^2	p -value	Nagelkerke's R^2
Inattention symptoms ^a	0.546 ± 0.178	9.445	.002	9.445	-0.275 ± 0.276	0.997	.932	.012
Hyperactive/impulsive symptoms ^a	0.112 ± 0.169	0.441	.506	.005	-0.01 ± 0.246	0.002	.967	.000

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, the effects of age, sex, handedness and DTI protocol were regressed out and the standardized residuals were used in the analyses.

Supplementary Table 3. Single regression analyses associating τ (estimated based on all nonerror trials of the sustained attention task) with the mean FA values of bilateral ROIs of SLF, ILF and CB corrected for age, sex, handedness, DTI protocol and the other ROIs

FA residuals*	Dependent variable: Tau (τ)			T_{195}	p -value	β	R^2
	N	$b \pm SE$					
Left SLF	196	1.082 ± 2.996		0.361	.718	.026	.001
Right SLF	196	-8.362 ± 2.936		2.936	.005	-.200	.040
Left ILF	196	-1.236 ± 2.996		-0.413	.68	-.030	.001
Right ILF	196	3.199 ± 2.988		1.070	.286	.077	.006
Left CB	196	1.454 ± 2.995		0.485	.628	.035	.001
Right CB	196	0.908 ± 2.996		0.303	.762	.022	.000

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

Supplementary Table 4. Single regression analyses associating τ (estimated based on all trials of the sustained attention task) with the mean FA values of bilateral ROIs of SLF, ILF and CB corrected for age, sex, handedness, DTI protocol and the other ROIs

FA residuals*	Dependent variable: Tau (τ)		T_{195}	p -value	β	R^2
	N	b \pm SE				
Left SLF	196	0.975 \pm 2.987	0.327	.744	.023	.001
Right SLF	196	-8.275 \pm 2.928	-2.826	.005	-.199	.040
Left ILF	196	-1.320 \pm 2.987	-0.442	.659	-.032	.001
Right ILF	196	3.308 \pm 2.979	1.111	.268	.079	.006
Left CB	196	1.519 \pm 2.986	0.509	.611	.037	.001
Right CB	196	0.784 \pm 2.987	0.263	.793	.019	.000

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

Supplementary Table 5. Single regression analyses associating τ with mean FA values of bilateral ROIs of SLF, ILF and CB corrected for age, sex, handedness, DTI protocol, the other ROIs and number of errors

FA residuals*	Dependent variable: Tau (τ)		T_{195}	p -value	β	R^2
	N	b \pm SE				
Left SLF	196	1.666 \pm 2.981	0.559	.577	.04	.002
Right SLF	196	-8.156 \pm 2.926	-2.788	.006	-.196	.039
Left ILF	196	-0.260 \pm 2.984	-0.087	.931	.006	.000
Right ILF	196	2.879 \pm 2.976	0.967	.335	.069	.005
Left CB	196	2.212 \pm 2.979	0.742	.459	.053	.003
Right CB	196	-0.840 \pm 2.983	-0.282	.779	-.020	.000

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

Supplementary Table 6. Regression analysis associating τ with mean FA values of bilateral regions of SLF, ILF and CB corrected for age, sex, handedness and DTI protocol

FA residuals*	ADHD patients (N = 100); dependent variable: Tau (τ)					Control (N = 96); dependent variable: Tau (τ)				
	b \pm SE	T_{99}	p -value	β	R^2	b \pm SE	T_{95}	p -value	β	R^2
Left SLF	6.475 \pm 4.093	1.582	.117	.158	.025	-5.321 \pm 3.696	-1.440	.153	-.15	.022
Right SLF	-13.023 \pm 3.997	-3.258	.002	-.313	.098	-2.118 \pm 3.661	-0.579	.564	-.06	.004
Left ILF	-1.632 \pm 4.427	-0.369	.713	-.713	.001	2.421 \pm 3.472	0.697	.487	.072	.005
Right ILF	4.773 \pm 4.761	0.1003	.318	.101	.01	.58 \pm 3.236	0.179	.858	.018	.000
Left CB	4.107 \pm 5.03	0.816	.416	.082	.007	-8.52 \pm 3.117	-0.273	.785	-.028	.001
Right CB	-1.715 \pm 4.688	-0.366	.715	-.037	.001	2.557 \pm 3.273	0.781	.437	.08	.006

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

Supplementary Table 7. Regression analyses associating diagnosis with mean FA values of bilateral regions of SLF, ILF and CB corrected for age, sex and handedness

FA residuals*	Dependent variable: diagnosis (group)					
	N	b(SE)	Wald- χ^2_1	p-value	β	Odds Ratio
Left SLF	196	-0.026 ± 0.147	0.033	.857	-.006	.974
Right SLF	196	-0.071 ± 0.147	0.234	.629	-.017	.931
Left ILF	196	-0.176 ± 0.148	1.404	.236	-.042	.839
Right ILF	196	0.099 ± 0.147	0.452	.501	.024	1.104
Left CB	196	0.091 ± 0.147	0.379	.538	.022	1.095
Right CB	196	-0.060 ± 0.147	0.167	.683	-.014	.942

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; OR = odds ratio; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

Supplementary Table 8. Regression analyses associating τ with mean FA values of bilateral regions of SLF, ILF and CB for the 2 scan protocols separately corrected for age, sex and handedness

FA residuals*	Protocol 1 (N = 136); dependent variable: Tau (τ)					Protocol 2 (N = 60); dependent variable: Tau (τ)				
	b ± SE	T ₁₃₆	p-value	β	R ²	b ± SE	T ₅₉	p-value	β	R ²
Left SLF	1.177 ± 3.473	0.339	.735	.029	.001	1.620 ± 5.837	0.278	.782	.036	.001
Right SLF	-8.391 ± 3.668	-2.288	.024	-.194	.038	-9.224 ± 4.715	-1.956	.055	-.249	.055
Left ILF	-1.015 ± 3.396	-0.299	.765	-.026	.001	-.014 ± 6.329	-0.002	.998	-.001	.001
Right ILF	3.669 ± 3.507	1.046	.297	.090	.008	1.277 ± 5.602	0.228	.821	.030	.001
Left CB	1.016 ± 3.571	0.285	.776	.025	.001	3.41 ± 5.372	0.635	.528	.083	.007
Right CB	1.585 ± 3.349	0.473	.637	.041	.002	-5.909 ± 6.652	-0.888	.378	-.116	.013

CB = cingulum bundle; DTI = diffusion tensor imaging; FA = fractional anisotropy; ILF = inferior longitudinal fasciculus; OR = odds ratio; ROI = region of interest; SE = standard error; SLF = superior longitudinal fasciculus.

*For each separate ROI, age, sex, handedness, DTI protocol and FA values of the other ROIs were regressed out and the standardized residuals were used in the analyses.

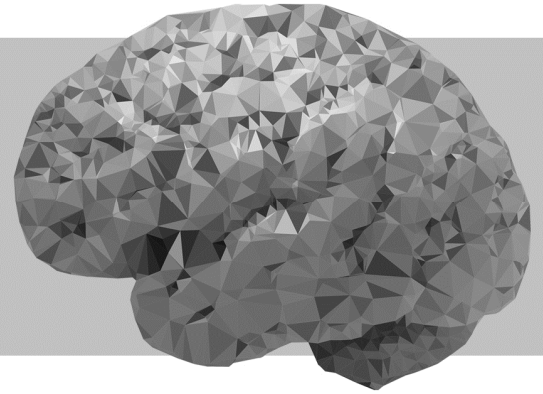
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Enlarged striatal volume in adults with ADHD carrying the 9-6 haplotype of the dopamine transporter gene *DAT1*

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Abstract

The dopamine transporter gene, *DAT1* (*SLC6A3*), has been studied extensively as a candidate gene for attention-deficit/hyperactivity disorder (ADHD). Different alleles of variable number of tandem repeats (VNTRs) in this gene have been associated with childhood ADHD (10/10 genotype and haplotype 10-6) and adult ADHD (haplotype 9-6). This suggests a differential association depending on age, and a role of *DAT1* in modulating the ADHD phenotype over the lifespan. The *DAT1* gene may mediate susceptibility to ADHD through effects on striatal volumes, where it is most highly expressed. In an attempt to clarify its mode of action, we examined the effect of three *DAT1* alleles (10/10 genotype, and the haplotypes 10-6 and 9-6) on bilateral striatal volumes (nucleus accumbens, caudate nucleus, and putamen) derived from structural magnetic resonance imaging scans using automated tissue segmentation. Analyses were performed separately in three cohorts with cross-sectional MRI data, a childhood/adolescent sample (NeuroIMAGE, 301 patients with ADHD and 186 healthy participants) and two adult samples (IMpACT, 118 patients with ADHD and 111 healthy participants; BIG, 1718 healthy participants). Regression analyses revealed that in the IMpACT cohort, and not in the other cohorts, carriers of the *DAT1* adult ADHD risk haplotype 9-6 had 5.9% larger striatum volume relative to participants not carrying this haplotype. This effect varied by diagnostic status, with the risk haplotype affecting striatal volumes only in patients with ADHD. An explorative analysis in the cohorts combined (N = 2434) showed a significant gene-by-diagnosis-by-age interaction suggesting that carriership of the 9-6 haplotype predisposes to a slower age-related decay of striatal volume specific to the patient group. This study emphasizes the need of a lifespan approach in genetic studies of ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset psychiatric disorder that features symptoms of age-inappropriate inattention and/or impulsivity and hyperactivity. ADHD affects 5-6% of children (Polanczyk et al., 2007) and frequently persists into adulthood (Faraone et al., 2006) causing a prevalence of ADHD of between 2.5% and 4.9% in the adult population (Simon et al., 2009). The heritability of ADHD is around 0.8 in both children (Faraone et al., 2005) and adults (Larsson et al., 2013). ADHD's complex genetic etiology likely involves multiple genes of small to moderate effect (Akutagawa-Martins et al., 2013).

The dopamine neurotransmission system has been an important focus of genetic research in ADHD, since it is the main site of action of stimulant drugs, the primary pharmacological treatment for the disorder (Cortese, 2012; Faraone et al., 2014a). One of the most appealing and extensively studied candidate genes for ADHD is the dopamine transporter (*DAT1*) gene (official name *SLC6A3*) (Faraone et al., 2005; Franke et al., 2012). The dopamine transporter is a key determinant of synaptic dopamine levels by regulating the reuptake of dopamine from the extracellular space, thereby terminating its synaptic action (Madras et al., 2005). The association between *DAT1* and ADHD was suggested in linkage and association studies and is confirmed in meta-analyses (Franke et al., 2010; Gizer et al., 2009; Li et al., 2006) showing small but significant effects on the susceptibility to ADHD. Meta-analyses of genetic association studies have indicated that the 10-repeat allele of the 3' untranslated region (UTR) variable number of tandem repeat (VNTR) is overrepresented in children with ADHD (Gizer et al., 2009). More recent studies suggested that the 10-repeat allele might increase ADHD risk in children particularly in the context of a haplotype with the 6-repeat allele of another VNTR in intron 8 of the gene (Asherson et al., 2007; Brookes et al., 2008). A recent study also found an association between this 10–6 haplotype and ADHD symptom measures in nonclinical adults (Tong et al., 2015), but association studies in clinical samples of adults with ADHD could not confirm this relationship (Brüggemann et al., 2007) and reported an association of the 9–6 haplotype with adult ADHD (Franke et al., 2008; Franke et al., 2010). Together, these findings suggest a role for *DAT1* in modulating the ADHD phenotype across the lifespan, with different associations depending on age and diagnostic status.

The specific mechanisms by which *DAT1* genetic variants affect the risk for ADHD are not well understood. Two imaging genetics studies showed that genetic variation of the *DAT1* gene is associated with altered striatal volume, which may contribute to ADHD susceptibility; the caudate nucleus, a sub-region of the striatum, was found to be smaller in children homozygous for the 10-repeat allele (10/10) than in carriers of the 9-repeat allele (Durstun et al., 2005; Shook et al., 2011). Although both studies did not find an interaction between presence/absence of ADHD and genotype, Durstun et al. (2005) reported that the effect of *DAT1* genotype on caudate volume was only significant in the subgroup of patients with ADHD. Studies investigating the effect of the *DAT1* gene on prefrontal gray matter volume, cortical thickness, or white matter integrity found no association between 10-repeat

allele carriers (10/10) and 9-repeat allele carriers (Durstun et al., 2005; Hong et al., 2015; Shaw et al., 2007), suggesting that this gene primarily affects regions, where it is highly expressed (i.e., the striatum) (Ciliax et al., 1999; Durstun et al., 2009).

The effect of the *DAT1* gene on striatal volumes may help explain smaller volumes of caudate nucleus and putamen typically found in children with ADHD (Ellison-Wright, Ellison-Wright, & Bullmore, 2008; Frodl & Skokauskas, 2012; Nakao, Radua, Rubia, & Mataix-Cols, 2011; Valera, Faraone, Murray, & Seidman, 2007). It has been shown that volumetric differences in caudate nucleus and the putamen gradually disappear with age (Castellanos et al., 2002; Frodl and Skokauskas, 2012; Greven et al., 2015; Maier et al., 2015; Nakao et al., 2011). The largest study to date by the ENIGMA ADHD Working Group containing 1713 participants with ADHD and 1529 controls show (among others) reduced accumbens, caudate nucleus, and putamen volume in ADHD. Case-control differences were most pronounced in childhood confirming a model of delayed brain growth and maturation (Hoogman et al., submitted). Nonetheless, there is evidence from studies of adults with persistent ADHD that differences in caudate nucleus volume (Almeida Montes et al., 2010; Onnink et al., 2014; Proal et al., 2011; Seidman et al., 2011; Shaw et al., 2014) and putamen volume (Seidman et al., 2011; Shaw et al., 2014) persist into adulthood.

To summarize, existing literature points to different alleles of the *DAT1* increasing susceptibility to categorically defined ADHD from childhood to adulthood, with a possible role of striatal volume in the pathway from gene to disease. The evidence for an influence of *DAT1* on striatal volume is based on relatively small-sampled studies (N = 59 in Shook et al. (2011) and N = 72 in Durstun et al. (2005)). Moreover, these studies examined only one variant of the *DAT1* gene (10/10 homozygotes versus 9-repeat carriers), not taking into account the potentially stronger effects of the two-VNTR haplotypes. Importantly, they were conducted in children only and could not test possible different effects of gene variation on striatal volume across the lifespan.

In the current study, we therefore set out to investigate the effects of the three different *DAT1* risk variants on striatal brain volume (nucleus accumbens, caudate nucleus, putamen) and the potential interaction with diagnostic status and age. We defined the *DAT1* 10/10 genotype, the 10-6 haplotype, and the 9-6 haplotype as risk alleles, based on associations with ADHD in children (10/10 genotype and 10-6 haplotype) and in adults (9-6 haplotype), respectively. Participants were derived from three cohorts with cross-sectional MRI data, a childhood/adolescent sample (NeuroIMAGE, 301 patients with ADHD and 186 healthy controls) and two adult samples (IMpACT, 118 patients with ADHD and 111 healthy controls; BIG, 1718 healthy participants).

Methods

Participants

Participants of this study were derived from three distinct cohorts. Ethical approval for all three was obtained, and all participants provided written informed consent.

A total of 487 subjects (301 unrelated patients with ADHD and 186 control participants) were derived from the NeuroIMAGE cohort of families with ADHD and control families (<http://www.neuroimage.nl>) (von Rhein et al., 2015). Only one individual per family was included thus (un)affected siblings were not included in this study. Participants were recruited at VU University Amsterdam, Amsterdam, and Radboud University Medical Center, Nijmegen. Inclusion criteria were an age between 8 and 30 years; European Caucasian descent; intelligence quotient (IQ) greater than or equal to 70; and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, and known genetic disorders. All participants were evaluated with a semi-structured diagnostic interview assessing ADHD, oppositional defiance disorder (ODD), and conduct disorder (CD). For further details on diagnostic assessment, see von Rhein et al. (2015).

A total of 229 subjects (118 adult patients with ADHD and 111 control participants) were included from the Dutch cohort of the International Multicentre persistent ADHD CollaboraTion, IMPACT (<http://www.impactadhdgenomics.com>; (Franke et al., 2010; Onnink et al., 2014). Participants were recruited at Radboud University Medical Center, Nijmegen. All participants were evaluated with semi-structured diagnostic interviews for assessing ADHD and axis I and axis II disorders. For details on diagnostic assessment, see Onnink et al. (2014). Inclusion criteria were an age between 18 and 65 years; European Caucasian descent; IQ greater than or equal to 70; no diagnosis of psychosis, alcohol or substance use disorder in the last 6 months, current major depression, neurological and sensorimotor disorders. An exclusion criterion for the control participants was a current neurological or psychiatric disorder.

A total of 1718 control participants were included from the Cognomics Initiative Resource, the Brain Imaging Genetics (BIG) study (<http://www.cognomics.nl>). This ongoing study started in 2007 and is a collection of healthy volunteers, many with a high education level, who participated in studies at the Donders Centre for Cognitive Neuroimaging (DCCN) of the Radboud University in Nijmegen (Guadalupe et al., 2014). The self-reported healthy individuals underwent anatomical (T1-weighted) magnetic resonance imaging (MRI) scans, usually as part of their involvement in diverse smaller-scale studies at the DCCN.

Genotyping

In all three cohorts, DNA was isolated from EDTA blood samples or saliva samples using standard procedures. Genotyping of the 40 base pair VNTR in the 3'UTR and the VNTR in intron 8 of *DAT1/SLC6A3* was carried out at the department of Human Genetics of the Radboud University Medical Center, Nijmegen as is described earlier (Franke et al., 2010). Haplotypes were calculated using the Haplostats package (Rversion 2.12.0) (Schaid et al., 2002).

Image Acquisition and Segmentation

MRI data in NeuroIMAGE were acquired at two locations (VU University Amsterdam, Amsterdam, and Radboud University Medical Center, Nijmegen) using two similar 1.5 Tesla

(T) scanners (Sonata and Avanto; Siemens Medical Systems, Erlangen, Germany) with closely matched scan protocols (von Rhein et al., 2015). MRI data in IMpACT were acquired with a 1.5T scanner (Avanto; Siemens Medical Systems, Erlangen, Germany). For NeuroIMAGE, GRAPPA2 (generalized autocalibrating partial parallel acquisition) and for IMpACT magnetization prepared rapid gradient echo sequence (MPRAGE) sequences were used. For NeuroIMAGE and IMpACT, all scans covered the entire brain and had a voxel size of 1x1x1 mm (176 sagittal slices; repetition time = 2730 milliseconds; echo time = 2.95 milliseconds; inversion time = 1000 milliseconds; flip angle = 7°; field of view = 256 mm). MRI data in BIG were acquired with either a 1.5T (Sonata and Avanto; Siemens Medical Systems, Erlangen, Germany) (N = 923) or with a 3T Siemens scanner (Trio and TimTrio; Siemens Medical Systems, Erlangen, Germany) (N = 796). Given that images were acquired during several smaller scale studies, the parameters used were slight variations of a standard T1-weighted sequence (MPRAGE; voxel size of 1x1x1 mm). The most common variations in the TR/TI/TE/sagittal-slices parameters were the following: 2300/1100/3.03/192, 2730/1000/2.95/176, 2250/850/2.95/176, 2250/850/3.93/176, 2250/850/3.68/176, 2300/1100/3.03/192, 2300/1100/2.92/192, 2300/1100/2.96/192, 2300/1100/2.99/192, 1940/1100/ 3.93/176 and 1960/1100/4.58/176. Such slight variations in these imaging parameters have been shown not to affect the reliability of morphometric results (Jovicich et al., 2009).

Whole-brain volume

Normalization, bias correction, and segmentation into gray matter, white matter, and cerebrospinal fluid volumes were performed using the unified procedure of the VBM 8.1 toolbox (<http://dbm.neuro.uni-jena.de/vbm/>) in SPM (default settings). Total gray and white matter volumes were calculated by summation of their tissue probability maps. Total brain volume was the sum of total gray and white matter volumes.

Striatal volumes

Automated FIRST (FMRIB's Integrated Registration and Segmentation Tool) subcortical segmentation was applied to estimate left and right hemisphere volumes of the nucleus accumbens, caudate nucleus, and putamen. The ENIGMA protocol (<http://enigma.ini.usc.edu/protocols/imaging-protocols/>) for the FIRST module (version 1.2) of FSL (version 4.1.5) was followed. FIRST is part of FMRIB's Software Library and performs registration and shape modeling of the just-mentioned regions in Montreal Neurological Institute 152 standard space (Patenaude et al., 2011). Total striatal volume was the sum of left and right volumes of the nucleus accumbens, caudate nucleus, and putamen.

Statistical analyses

Brain volumetric measures were normally distributed, and outliers defined as more than three standard deviations greater than or less than the mean were removed. Overall, there were few outliers (1-5 individuals per volume). For each cohort independently, the effect of

three variants of the *DAT1* gene on striatal volumes were examined by comparing: 1) carriers of the 10/10 genotype with all non-carriers, 2) carriers of at least one copy of the 10-6 haplotype with all non-carriers, and 3) carriers of at least one copy of the 9-6 haplotype with all non-carriers. Associations between the three risk variants of the *DAT1* gene and striatal volumes were examined using regression analyses in SPSS (IBM SPSS v.20). Regression analyses included variant of the *DAT1* gene, diagnostic status, and the interaction between risk variant and diagnostic status (*DAT1* variant * diagnostic status) as predictors and total striatal volume as dependent measure. Included covariates were age, gender, and total brain volume (sum of white and gray matter); for the NeuroIMAGE and BIG cohorts, additional covariates were scanner location and type (for NeuroIMAGE: Amsterdam or Nijmegen; for BIG: 1.5T or 3.0T); for the BIG cohort with healthy participants, diagnostic status was dropped from the model. Centering of variables was used (Bradley and Srivastava, 1979). First, we tested the interaction between *DAT1* variant and diagnostic status. Whenever this interaction term was significant ($p < .05$), we analyzed the results separately by diagnostic status. If not significant, this interaction was dropped from the model. For significant main effects of the three risk variants, we performed *post-hoc* sensitivity analyses. Correcting with covariates in a regression analysis is only appropriate if covariate means or distributions are equal between groups (Miller and Chapman, 2001). Therefore, sensitivity analyses in a matched subsample were performed for the instances in which covariates differed between groups. Automatic case-control matching was performed with the FUZZY extension for SPSS (<http://www.spss.com/devcentral>). Sensitivity analyses were performed to investigate the effect of the risk variant on each subregion of the striatum (left and right volumes of nucleus accumbens, caudate nucleus, and putamen). Additionally, we investigated the possible effect of medication on the results by including lifetime medication use (yes or no) to the model. To explore potential interactions between *DAT1* variant, diagnostic status, and age on striatal volume (*DAT1* variant * diagnostic status * age), we combined the samples from the three cohorts into one sample in order to maximize the age range. Then, striatal volume was adjusted for the same covariates as mentioned above, except age, using a linear regression analysis from which standardized residuals were computed and were used in the analyses (Walhovd et al., 2005). To visualize potential age effects, the residuals were also plotted.

Correction for multiple testing

To correct for multiple testing, Bonferroni correction was applied by dividing the significance level by the number of independent tests. In three cohorts (NeuroIMAGE, IMpACT, BIG), we examined the effects of three alleles/genotypes (10/10, 10-6 haplotype, 9-6 haplotype) on striatal volume. We performed a total of nine tests and set the multiple-testing adjusted p -value at $0.05/9 = 0.0055$. *Post-hoc* sensitivity analyses of findings surviving multiple-testing correction used the nominal significance level ($p < .05$).

Results

Demographics

Demographics for ADHD patients and control participants are displayed for the NeuroIMAGE, IMpACT, and BIG cohorts separately in Table 1. From the NeuroIMAGE cohort, the 301 patients with ADHD and 186 control participants were evenly distributed across groups based on VNTR genotypes (10/10) and *DAT1* haplotypes (10-6 haplotype or 9-6 haplotype). In this cohort, patients were significantly older compared with the control participants ($t(1, 485) = 2.21, p = .03$), and gender distribution was significantly different, with males predominating in the ADHD group and females in the control group ($\chi^2 = 16.19, p < .001$). From the IMpACT cohort, 118 patients with ADHD and 111 control participants were included, for which no differences in the distribution of *DAT1* 10/10 genotype and *DAT1* 10-6 haplotype were observed. The 9-6 haplotype showed a higher prevalence in patients compared with controls ($\chi^2 = 5.21, p = .023$; see Table 1), as was reported previously in this cohort (Hoogman et al., 2012). From the BIG cohort, 1718 healthy participants were included. Genotype distributions did not deviate from Hardy-Weinberg Equilibrium, and frequencies were as expected in Caucasian samples (Franke et al., 2010).

Demographics for 10/10, 10-6, and 9-6 carrier and respective non-carrier groups are displayed for the NeuroIMAGE, IMpACT, and BIG cohorts separately in Supplementary Tables 1, 2, and 3. In the IMpACT cohort, gender distribution was significantly different between *DAT1* 10/10 carriers and non-carriers ($\chi^2 = 4.47, p = .03$; Supplementary Table 1), with males predominating in the *DAT1* 10/10 group and females in the non-*DAT1* 10/10 group. Gender distribution was also significantly different between *DAT1* 9-6 carriers and non-carriers ($\chi^2 = 5.16, p = .02$; Supplementary Table 3), with males predominating in the *DAT1* 9-6 group and females in the non-carriers.

Table 1. Participant characteristics for the three cohorts included in this study

	NeuroIMAGE (N = 487)		Test of significance	IMpACT (N = 229)		Test of significance	BIG (N = 1718)
Characteristics	ADHD (N = 301)	Controls (N = 186)		ADHD (N = 118)	Controls (N = 111)		Controls (N = 1718)
10/10 carriers, N (%)	183 (61)	105 (56)	$\chi^2 = 0.90, p = .34$	61 (52)	69 (62)	$\chi^2 = 2.55, p = .11$	978 (57)
10-6 carriers, N (%)	282 (94)	174 (94)	$\chi^2 = 0.01, p = .95$	107 (91)	102 (92)	$\chi^2 = 0.11, p = .75$	1573 (92)
9-6 carriers, N (%)	49 (16)	24 (13)	$\chi^2 = 1.03, p = .31$	26 (22)	12 (11)	$\chi^2 = 5.21, p = .02$	249 (14)
Male, N (%)	207 (69)	94 (51)	$\chi^2 = 16.19, p < .001$	46 (39)	46 (41)	$\chi^2 = 0.14, p = .71$	749 (44)
Age in years, mean (SD)	17.21 (3.27)	16.55 (3.06)	$t(1, 485) = -2.21, p = .03$	35.94 (10.93)	37.03 (11.28)	$t(1, 227) = 0.72, p = .47$	26.06 (10.63)
IQ, mean (SD)	97.02 (15.24)	106.39 (13.38)	$t(1, 485) = 6.89, p < .001$	107.81 (14.50)	110.03 (15.41)	$t(1, 227) = 1.12, p = .26$	nd
Inattentive scale, mean (SD) ^a	65.89 (11.09)	46.28 (5.70)	$t(1, 485) = -22.27, p < .001$	6.46 (2.04)	0.66 (1.12)	$t(1, 227) = -26.81, p < .001$	1.20 (1.66)
Hyperactive/i mpulsive scale, mean (SD) ^a	69.63 (14.45)	46.28 (5.01)	$t(1, 485) = -21.19, p < .001$	5.48 (2.24)	0.90 (1.38)	$t(1, 227) = -18.49, p < .001$	1.62 (1.65)
Total brain volume in ml, mean (SD) ^b	1257.73 (125.41)	1265.41 (123.03)	$t(1, 485) = 0.61, p = .51$	1255.06 (106.58)	1240.83 (124.09)	$t(1, 227) = -0.93, p = .35$	123.90 (120.10)

^a For NeuroIMAGE cohort: measured with the Conners' Parent Rating Scale–Revised (Conners et al., 1998). Values refer to *t* scores on the *DSM* Total, Inattentive Behavior, and Hyperactive-Impulsive Behavior scales (scales N, L, and M). For IMpACT and BIG cohorts: measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^b Total brain volume is defined as the sum of total gray and white matter.

nd = not determined

Main and interaction effects of *DAT1* variants on total striatum volume

For each cohort, mean total striatum volumes corrected for covariates are shown in Table 2. In the IMpACT cohort, subjects carrying at least one copy of the 9-6 risk haplotype showed a 5.9% larger striatum volume (1.09 ml larger) than subjects carrying none ($\beta = 1.09$; 95% CI 0.63 to 1.56; $p = .00001$) (Table 2 and 3). No effects of the *DAT1* variant (combinations) were observed in the NeuroIMAGE or BIG cohorts.

In the IMpACT cohort, an interaction between the *DAT1* 9-6 haplotype and diagnostic status on striatal volume was significant ($p = .02$). Testing patients with ADHD and controls separately revealed that patients carrying at least one copy of the *DAT1* 9-6 haplotype had larger striatum volume (7.4%; 1.37 ml; $\beta = 1.37$; 95% CI 0.80 to 1.94; $p = .00001$), while this effect was not significant in the control group (3.0%; 0.57 ml; $\beta = 0.57$; 95% CI -0.25 to 1.39; $p = .17$) (Table 3 and Supplementary Table 5). Another significant interaction also observed in the IMpACT cohort was between diagnostic status and *DAT1* 10/10 genotype ($p = .005$). Post-hoc analyses revealed that patients homozygous for the 10R allele (10/10 carriers) had smaller striatum volume than 9R carriers (-3.5%; 0.64 ml; $\beta = -0.64$; 95% CI -1.14 to -0.14; $p =$

.013), while this effect was not present in the control group (1.4%; 0.36 ml; $\beta = 0.36$; 95% CI - 0.17 to 0.89; $p = .18$) (Table 3 and Supplementary Table 4).

Table 2. Total striatum volume for risk and non-risk carriers of specific *DAT1* variants. Volumes are also shown for controls and ADHD patients, separately

	Total striatum volume (ml)						
	NeuroIMAGE (N = 487)			IMpACT (N = 229)			BIG (N = 1718)
	Overall ^a	Controls ^b	ADHD ^b	Overall ^a	Controls ^b	ADHD ^b	Overall ^b
	Mean (SE), N	Mean (SE), N	Mean (SE), N	Mean (SE), N	Mean (SE), N	Mean (SE), N	Mean (SE), N
10/10 carriers	20.23 (0.09), 288	20.11 (0.13), 105	20.33 (0.10), 183	18.84 (0.12), 130	19.10 (0.16), 69	18.55 (0.17), 61	19.42 (0.04), 977
Non-carriers	20.01 (0.10), 199	20.15 (0.15), 81	19.96 (0.13), 118	19.01 (0.14), 99	18.74 (0.21), 42	19.19 (0.18), 57	19.45 (0.05), 741
10-6 carriers	20.11 (0.07), 456	20.11 (0.10), 174	20.16 (0.08), 282	18.87 (0.09), 209	19.11 (0.45), 102	18.81 (0.13), 107	19.44 (0.03), 1572
Non-carriers	20.46 (0.25), 31	20.32 (0.39), 12	20.60 (0.32), 19	19.28 (0.31), 20	18.95 (0.13), 9	19.34 (0.43), , 11	19.42 (0.10), 146
9-6 carriers	19.95 (0.17), 73	20.02 (0.27), 24	20.01 (0.21), 49	19.83 (0.22), 38	19.47 (0.38), 12	19.93 (0.25), 26	19.49 (0.08), 249
Non-carriers	20.17 (0.07), 414	20.14 (0.11), 162	20.22 (0.09), 301	18.73 (0.09), 191	18.90 (0.13), 99	18.56 (0.13), 92	19.43 (0.03), 1469

^a Means are based on estimated marginal means corrected for diagnostic status, age, gender, total brain volume; for the NeuroIMAGE cohort, covariates also included scanner type.

^b Means are based on estimated marginal means corrected for age, gender, total brain volume; for the NeuroIMAGE and BIG cohorts, covariates also included scanner type/location.

Total striatum volume is the sum of total left and right nucleus accumbens, caudate nucleus, and putamen volumes.

Boldface indicates results surviving multiple-testing correction.

Table 3. Regression of binary genotypes on total striatal volume^a

	NeuroIMAGE (N = 487)	IMpACT (N = 229)	BIG (N = 1718)
	β (95% CI), <i>p</i> -value ^b	β (95% CI), <i>p</i> -value ^b	β (95% CI), <i>p</i> -value ^b
<i>DAT1</i> 10/10	0.22 (-0.04;0.48), .09	-0.16 (-0.53;0.20), .38	-0.03 (-0.15;0.09), .57
Diagnostic status	0.22 (-0.05;0.49), .12	-0.29 (-0.62;0.07), .11	
Diagnostic status * <i>DAT1</i> 10/10	ns	-1.03 (-1.74;-0.32), .005	
<i>DAT1</i> 10-6	-0.35 (-0.86;0.16), .18	-0.41 (-1.04;0.22), .21	0.02 (-0.19;0.24), .84
Diagnosis	0.24 (-0.34;0.51), .09	-0.28 (-0.63;0.08), .13	
Diagnostic status * <i>DAT1</i> 10-6	ns	ns	
<i>DAT1</i> 9-6	-0.22 (-0.57;0.13), .21	1.09 (0.63;1.56), .00001	0.06 (-0.11;0.23), .47
Diagnosis	0.24 (-0.33;0.51), .09	-0.40 (-0.74;-0.05), .024	
Diagnostic status * <i>DAT1</i> 9-6	ns	1.14 (0.17;2.11), .021	

^a Results from the final regression model examining associations between binary genotype (risk carriers vs non-risk carriers) and brain volumes. Boldface indicates results surviving multiple-testing correction.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were diagnostic status, age, gender, total brain volume; for the NeuroIMAGE and BIG cohorts, covariates also included scanner type; for the BIG cohort, diagnosis was dropped from the model.

^c $\beta = 1.09$ denotes that 9-6 carriers had a 1.09 ml larger striatum volume than non 9-6 carriers.

For the NeuroIMAGE and IMpACT cohorts, interactions with genotype and diagnostic status (genotype * diagnostic status) were tested and removed when not nominal significant ($p < .05$).

ns = not significant

Sensitivity analyses

In the NeuroIMAGE cohort, gender distribution and age were significantly different between patients and controls (Table 1). We therefore examined the effect of the three variants of the *DAT1* gene on striatal volume in a subsample that was matched for gender and age (Supplementary Table 6). The results in this matched subsample (Supplementary Table 7) supported the results of the unmatched sample (Table 3). In the IMpACT cohort, gender distribution was significantly different between *DAT1* 10/10 carriers and non-carriers (Supplementary Table 1) and between *DAT1* 9-6 carriers and non-carriers (Supplementary Table 3). However, analysis of the effects of these two *DAT1* variants on striatal volume in a gender-matched subsample (Supplementary Table 8) confirmed the results observed in the full sample (Supplementary Table 9 and Table 3). The effect of the *DAT1* 9-6 haplotype on striatal volume found in the IMpACT cohort was the strongest effect observed, surviving multiple-testing correction, and was investigated further. Sensitivity analyses in the IMpACT cohort were performed to examine the effect of the *DAT1* 9-6 haplotype on the six subregions of the striatum independently (left and right volumes of nucleus accumbens, caudate nucleus, and putamen). Compared to subjects carrying no copies of the 9-6 risk haplotype, subjects carrying at least one copy of the risk haplotype had larger right putamen volume (6.2%; 0.33 ml; $\beta = 0.33$; 95% CI 0.17 to 0.48; $p = .00005$), larger left putamen (6.1%; 0.32 ml; $\beta = .32$; 95% CI 0.14 to 0.49; $p = .0004$), larger right caudate nucleus (5.9%; 0.22 ml; $\beta = 0.22$; 95% CI 0.09 to 0.35; $p = .001$), larger left caudate nucleus (5.5%; 0.20 ml; $\beta = 0.20$;

95% CI 0.07 to 0.33; $p = .002$), and larger right nucleus accumbens (5.8%; 0.03 ml; $\beta = 0.03$; 95% CI 0.01 to 0.06; $p = .04$). Findings were not significant for left nucleus accumbens ($p > .05$) (Supplementary Table 10). Testing the effect of the *DAT1* 9-6 haplotype on the six subregions of the striatum for patients with ADHD and controls separately revealed similar results as above in the patients, while effects were non-significant in controls (all p -values $> .05$) (Supplementary Table 11). Furthermore, rerunning analyses including medication use (yes or no) in the model yielded highly similar results (Supplementary Table 12).

Age effects of the *DAT1* 9-6 haplotype

To explore potential interactions between the *DAT1* 9-6 haplotype, diagnostic status, and age on total striatum volume, we combined the samples from the three cohorts into one sample in order to maximize the age range. Total striatal volume was regressed on covariates of no interest and the standardized residuals were used for analysis. In this mega-analysis design, the 3-way interaction between the 9-6 haplotype, diagnostic status, and age on striatal volume was significant ($p = .0001$). Testing patients with ADHD and controls separately revealed that the interaction between *DAT1* 9-6 haplotype and age was significant in the patient group ($p = .00004$) but not in the control group ($p = .94$) (Fig. 1).

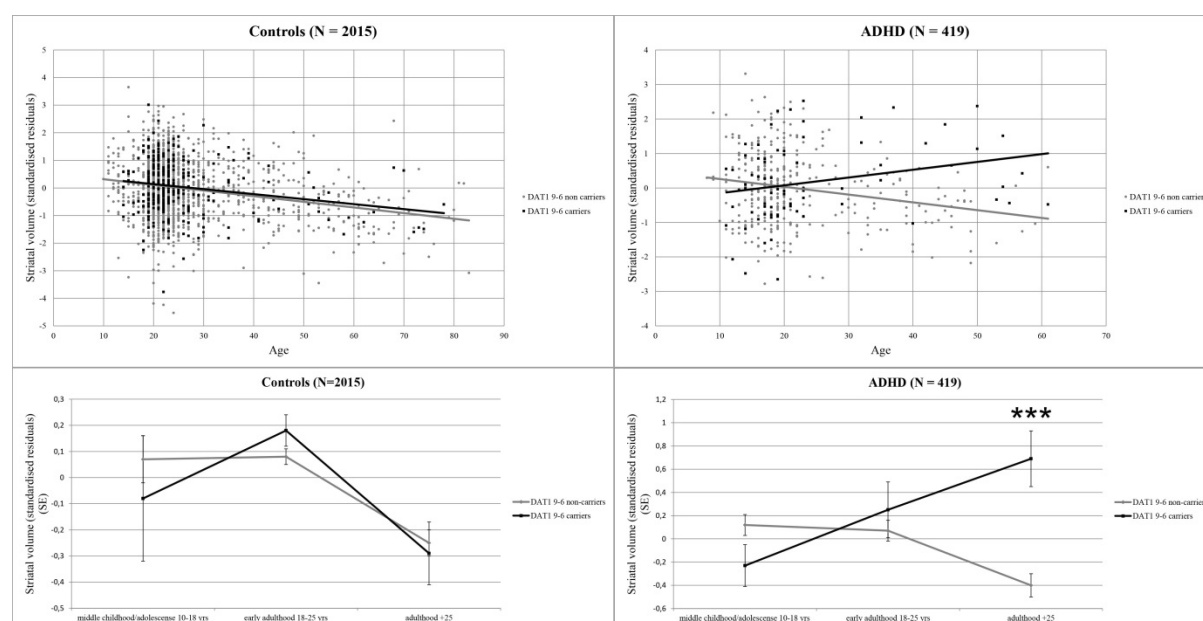


Figure 1. Age-related changes in the striatal volume. a) Regression plots visualizing the 3-way interaction (*DAT1* genotype * diagnostic status * age) by plotting the relationships between age and total striatal volume for *DAT1* 9-6 haplotype carriers and non-carriers separately for controls and ADHD patients. b) Same data as in Figure 1a although now visualized using separate age groups. The figures suggest that carriership of the 9-6 haplotype predisposes to a slower age-related decay of striatal volume in patients with ADHD.

Discussion

In the current study, the effect of the dopamine transporter gene *DAT1/SLC6A3* on striatal brain volume was investigated in children and adults with ADHD and healthy participants in three different cross-sectional cohorts. In the adult case-control cohort IMpACT, carriers of the 9-6 haplotype, the risk allele for adult ADHD, had larger striatal volume than participants

not carrying this haplotype. This effect varied by diagnostic group, with the risk haplotype affecting striatal volumes only in patients with ADHD and not in the healthy participants from this cohort. Consistent with this, the effect was not found in the BIG cohort of adult healthy participants. It was also not observed in the case-control children/adolescents cohort from NeuroIMAGE. Through an interaction analysis within the IMpACT cohort, also the 10/10 genotype was shown to affect striatal volume in patients only when compared to carriers of 9R allele(s), which was a smaller effect than for the 9-6 haplotype (and probably was just the other side of the same coin).

The finding in the IMpACT cohort showing smaller striatal volume in adult ADHD patients homozygous for the 10R allele (10/10 carriers) compared to 9R carriers is consistent with previous studies performed in children (Durstan et al., 2005; Shook et al., 2011). However, as 84% of the 9R carriers consisted of 9-6 haplotype carriers, this effect might be driven by the subgroup of 9-6 haplotype carriers. Indeed, the regression coefficient of -0.64 ($p = .013$, $N = 118$) (Supplementary Table 4) dropped to -0.074 ($p = .78$, $N = 92$) when the 9-6 haplotype carriers ($N = 26$) were excluded from the analysis (data not shown). The diagnosis-specificity of *DAT1* only affecting striatal volume in the subgroup of patients with ADHD was also suggested in the previous study by Durston et al. (2005). Larger striatal volume in adult carriers of the *DAT1* risk haplotype 9-6 for adult ADHD may represent compensatory mechanisms for the increased expression/activity of the dopamine transporter, which has been found in 9-repeat allele carriers (Faraone et al., 2014b). The increased levels of DAT in these individuals might lead to more efficient clearing of extracellular dopamine, yielding lower extracellular levels and reduced dopamine signaling (Faraone et al., 2014b). Importantly, a study by Spencer and coworkers showed that an ADHD diagnosis made an additional, independent contribution to DAT binding (Spencer et al., 2013). The diagnosis-specificity of our findings may thus reflect an interaction between genetic and environmental risk factors, where cumulative effects allow for a bigger impact of *DAT1* genotype on striatal volume in the patients. We emphasize, nonetheless, that replication of our findings is needed before firm conclusions can be drawn.

Our explorative 3-way interaction analysis in the cohorts combined ($N = 2434$) investigating the effect *DAT1* 9-6 haplotype, diagnostic status, and age suggests that carriership of the 9-6 haplotype predisposes to a slower age-related decay of striatal volume, which is specific for ADHD patients (Fig. 1). Importantly, age effects have shown a differential decay of *DAT1* expression for different genotypes (Shumay et al., 2011), which may be consistent with the compensation hypothesis mentioned above. Shumay et al. demonstrated that 9-repeat homozygotes showed the steepest decline of DAT availability with increasing age. Great care is needed in interpreting the age effects we observed, as this is a cross-sectional study. Interestingly, a recent study suggests that individuals can meet symptom criteria for ADHD as adults without having a history of childhood ADHD (Moffitt et al., 2015). Although this study by Moffitt et al. is in need of replication, our results may suggest that carriership of the *DAT1* 9-6 haplotype might be a mechanism contributing

to the emergence of new cases of ADHD during adulthood. However, to replicate our age-dependent effect and to explore this more fully, analysis of longitudinal MRI data is required.

The functional implications of larger striatal volume for the pathophysiology of adult ADHD remain to be investigated. As smaller caudate volume in male patients with ADHD has been associated with an increased number of hyperactivity/impulsivity symptoms (Onnink et al., 2014), larger striatum volume in a subgroup of ADHD patients may be linked to neurobiological processes that go along with the reported age-dependent decline in hyperactivity/impulsivity symptoms in people with ADHD (Biederman et al., 2000). Increased volume may also reflect compensatory ‘hypertrophy’ because of reduced dopamine neurotransmission (see above).

Our findings should be viewed in the light of certain strengths and limitations. A clear strength was the investigation of haplotypes of *DAT1* in addition to the 3’UTR VNTR genotype variants in a large sample including patients with ADHD and healthy individuals at different ages. This case-control design maximized the variance in the phenotype and may have magnified gene effects. A strong limitation was the cross-sectional MRI study design, especially since the participants of this study were partly derived from different cohorts. Another limitation was the restricted availability of data at early childhood age and late adult age, which reflects insufficient focus of imaging research in our field on such age groups. The developmental trajectories our data propose need to be confirmed in additional studies, optimally from longitudinal studies including data across a wide age range collected using the same study protocol.

In summary, our cross-sectional findings showed that adult patients with ADHD carrying the *DAT1* 9-6 risk haplotype for adult ADHD had increased striatal volume. Furthermore, based on our exploratory analysis on age effects, we hypothesize that ADHD patients carrying the 9-6 haplotype follow a different trajectory of brain development over the lifespan than those ADHD patients not carrying this haplotype. These findings are in need of replication, preferably using longitudinal designs. Clarifying the nature of the involvement of *DAT1* variants in brain development would provide a key step towards understanding part of ADHD’s pathophysiology. The present results demonstrate the importance of taking into account interindividual variability, as indexed by *DAT1* haplotype, presence of an ADHD diagnosis, and age, when assessing striatal volume effects in ADHD.

Supplementary information

Supplementary Table 1. Participant characteristics for the *DAT1* 10/10 carriers and non-carriers for the three cohorts included in this study

Characteristics	NeuroIMAGE (N = 487)			IMpACT (N = 229)			BIG (N = 1718)		
	<i>DAT1</i> 10/10 carriers (N = 288)	<i>DAT1</i> 10/10 non-carriers (N = 199)	Test of significance	<i>DAT1</i> 10/10 carriers (N = 130)	<i>DAT1</i> 10/10 non-carriers (N = 99)	Test of significance	<i>DAT1</i> 10/10 carriers (N = 977)	<i>DAT1</i> 10/10 non-carriers (N = 741)	Test of significance
Male, N (%)	167 (58)	134 (67)	$\chi^2 = 4.36, p = .05$	60 (46)	32 (32)	$\chi^2 = 4.47, p = .03$	416 (43)	333 (45)	$\chi^2 = 0.95, p = .33$
Age in years, mean (SD)	16.99 (3.29)	16.91 (3.08)	$t(1, 485) = -0.26, p = .80$	35.77 (10.95)	37.38 (12.29)	$t(1, 227) = 1.07, p = .29$	26.33 (11.19)	25.71 (9.84)	$t(1, 1716) = 1.20, p = .23$
IQ, mean (SD)	100.95 (15.47)	100.13 (14.92)	$t(1, 485) = 0.59, p = .56$	109.73 (14.84)	107.78 (15.11)	$t(1, 227) = 0.98, p = .33$	n.d.	n.d.	n.d.
Inattentive scale, mean (SD) ^a	58.74 (13.89)	57.69 (12.62)	$t(1, 485) = 0.84, p = .40$	3.31 (3.26)	4.09 (3.41)	$t(1, 227) = -1.76, p = .08$	1.18 (1.66)	1.23 (1.66)	$t(1, 972) = -0.42, p = .67$
Hyperactive/impulsive scale, mean (SD) ^a	60.99 (16.58)	60.10 (16.02)	$t(1, 485) = 0.59, p = .56$	2.96 (2.75)	3.66 (3.19)	$t(1, 227) = -1.77, p = .08$	1.73 (1.69)	1.47 (1.58)	$t(1, 972) = -2.38, p = .02$
Total brain volume in ml, mean (SD) ^b	1251.62 (123.39)	1273.74 (123.39)	$t(1, 485) = 1.93, p = .79$	1239.32 (111.98)	1254.89 (117.86)	$t(1, 227) = -1.01, p = .31$	1243.85 (121.05)	1233.38 (119.25)	$t(1, 1716) = 1.78, p = .07$

^a For NeuroIMAGE cohort: measured with the Conners' Parent Rating Scale-Revised (Conners et al. 1998). Values refer to *t* scores on the *DSM* Total, Inattentive Behavior, and Hyperactive-Impulsive Behavior scales (scales N, L, and M). For IMpACT and BIG cohorts: measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^b Total brain volume is defined as the sum of total gray and white matter.

nd = not determined

Supplementary Table 2. Participant characteristics for the *DAT1* 10-6 carriers and non-carriers for the three cohorts included in this study

Characteristics	NeuroIMAGE (N = 487)			IMpACT (N = 229)			BIG (N = 1718)		
	<i>DAT1</i> 10-6 carriers (N = 456)	<i>DAT1</i> 10-6 non-carriers (N = 31)	Test of significance	<i>DAT1</i> 10-6 carriers (N = 209)	<i>DAT1</i> 10-6 non-carriers (N = 20)	Test of significance	<i>DAT1</i> 10-6 carriers (N = 1575)	<i>DAT1</i> 10-6 non-carriers (N = 143)	Test of significance
Male, N (%)	283 (62)	18 (58)	$\chi^2 = 0.20, p = .66$	87 (42)	5 (25)	$\chi^2 = 0.96, p = .34$	689 (44)	60 (42)	$\chi^2 = 0.17, p = .68$
Age in years, mean (SD)	16.95 (2.22)	16.95 (3.26)	$t(1, 485) = -0.01, p = .99$	36.69 (11.35)	34.15 (11.43)	$t(1, 227) = 0.72, p = .47$	26.15 (10.65)	25.15 (10.42)	$t(1, 1716) = 1.07, p = .29$
IQ, mean (SD)	100.68 (15.31)	99.68 (14.36)	$t(1, 485) = 0.35, p = .72$	108.66 (15.12)	111.34 (13.26)	$t(1, 227) = 0.77, p = .45$	n.d.	n.d.	n.d.
Inattentive scale, mean (SD) ^a	58.39 (13.52)	57.23 (11.37)	$t(1, 485) = 0.46, p = .65$	3.60 (3.33)	4.15 (3.54)	$t(1, 227) = -0.70, p = .48$	1.18 (1.65)	1.42 (1.74)	$t(1, 972) = 1.28, p = .20$
Hyperactive/impulsive scale, mean (SD) ^a	60.93 (16.48)	56.18 (13.67)	$t(1, 485) = 1.55, p = .12$	3.27 (3.27)	3.15 (3.13)	$t(1, 227) = 0.17, p = .86$	1.62 (1.67)	1.58 (1.43)	$t(1, 972) = -0.25, p = .80$
Total brain volume in ml, mean (SD) ^b	1260.73 (112.81)	1260.66 (125.34)	$t(1, 485) = 0.01, p = .99$	1247.52 (112.20)	1254.89 (147.74)	$t(1, 227) = -0.27, p = .79$	1247.78 (128.22)	1237.00 (119.34)	$t(1, 972) = 1.03, p = .30$

^a For NeuroIMAGE cohort: measured with the Conners' Parent Rating Scale-Revised (Conners et al. 1998). Values refer to *t* scores on the *DSM* Total, Inattentive Behavior, and Hyperactive-Impulsive Behavior scales (scales N, L, and M). For IMpACT and BIG cohorts: measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^b Total brain volume is defined as the sum of total gray and white matter.

nd = not determined

Supplementary Table 3. Participant characteristics for the *DAT1* 9-6 carriers and non-carriers for the three cohorts included in this study

Characteristics	NeuroIMAGE (N = 487)			IMpACT (N = 229)			BIG (N = 1718)		
	<i>DAT1</i> 9-6 carriers (N = 73)	<i>DAT1</i> 9-6 non-carriers (N = 414)	Test of significance	<i>DAT1</i> 9-6 carriers (N = 38)	<i>DAT1</i> 9-6 non-carriers (N = 191)	Test of significance	<i>DAT1</i> 9-6 carriers (N = 249)	<i>DAT1</i> 9-6 non-carriers (N = 1469)	Test of significance
Male, N (%)	50 (68)	251 (61)	$\chi^2 = 1.62, p = .20$	9 (24)	83 (43)	$\chi^2 = 5.16, p = .02$	101 (41)	648 (44)	$\chi^2 = 1.09, p = .30$
Age in years, mean (SD)	17.12 (2.88)	16.93 (3.26)	$t(1, 485) = -0.47, p = .64$	37.66 (13.57)	36.23 (10.89)	$t(1, 227) = -1.02, p = .30$	25.69 (10.42)	26.13 (10.67)	$t(1, 1716) = 0.61, p = .55$
IQ, mean (SD)	98.13 (14.39)	101.05 (15.36)	$t(1, 485) = 1.50, p = .13$	111.19 (15.32)	108.43 (14.88)	$t(1, 227) = 0.98, p = .33$	n.d.	n.d.	n.d.
Inattentive scale, mean (SD) ^a	58.11 (12.08)	58.13 (13.62)	$t(1, 485) = 0.14, p = .89$	4.92 (3.40)	3.39 (3.28)	$t(1, 227) = -2.61, p = .01$	1.10 (1.46)	1.22 (1.69)	$t(1, 972) = 0.78, p = .44$
Hyperactive/impulsive scale, mean (SD) ^a	59.84 (14.79)	60.77 (16.62)	$t(1, 485) = 0.45, p = .65$	4.42 (3.19)	3.03 (2.87)	$t(1, 227) = -2.68, p = .008$	1.35 (1.58)	1.66 (1.66)	$t(1, 972) = -2.07, p = .04$
Total brain volume in ml, mean (SD) ^b	1270.32 (119.62)	1258.96 (125.33)	$t(1, 485) = -0.72, p = .47$	1226.00 (101.37)	1252.57 (117.69)	$t(1, 227) = 1.22, p = .20$	1237.01 (119.60)	1238.05 (120.23)	$t(1, 1716) = 2.07, p = .04$

^a For NeuroIMAGE cohort: measured with the Conners' Parent Rating Scale-Revised (Conners et al. 1998). Values refer to *t* scores on the *DSM* Total, Inattentive Behavior, and Hyperactive-Impulsive Behavior scales (scales N, L, and M). For IMpACT and BIG cohorts: measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^b Total brain volume is defined as the sum of total gray and white matter.

nd = not determined

Supplementary Table 4. Striatal volumes for *DAT1* 10/10 carriers and non-carriers for ADHD patients and controls separately from the IMpACT cohort

IMpACT ADHD (N = 118)			
	<i>DAT1</i> 10/10 carriers (N = 61)	<i>DAT1</i> 10/10 non-carriers (N = 57)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	18.55 (0.17)	19.19 (0.18)	-0.64; (-1.14;-0.14), .013
IMpACT Controls (N = 111)			
	<i>DAT1</i> 10/10 carriers (N = 69)	<i>DAT1</i> 10/10 non-carriers (N = 42)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	19.10 (0.16)	18.73 (0.21)	0.36 (-0.17;.89), .18

^a Means are based on estimated marginal means corrected for age, gender, and total brain volume.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were diagnostic status, age, gender and, total brain volume.

Supplementary Table 5. Striatal volumes for *DAT1* 9-6 carriers and non-carriers for ADHD patients and controls separately from the IMpACT cohort

IMpACT ADHD (N = 118)			
	<i>DAT1</i> 9-6 carriers (N = 26)	<i>DAT1</i> 9-6 non-carriers (N = 92)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	19.93 (0.25)	18.56 (0.13)	1.37 (0.80;1.94), .00001
IMpACT Controls (N = 111)			
	<i>DAT1</i> 9-6 carriers (N = 26)	<i>DAT1</i> 9-6 non-carriers (N = 85)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	19.47 (0.39)	18.90 (0.13)	0.57 (-0.25;1.39), .17

^a Means are based on estimated marginal means corrected for age, gender, and total brain volume.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were age, gender, and total brain volume.

Boldface indicates results surviving multiple-testing correction.

Supplementary Table 6. Participant characteristics for ADHD and control subjects from the NeuroIMAGE cohort, matched for gender and age

Characteristics	NeuroIMAGE (N = 487)		Test of significance
	ADHD (N = 301)	Controls (N = 186)	
10/10 carriers, N (%)	117 (53)	104 (47)	$\chi^2 = 1.96, p = .16$
10-6 carriers, N (%)	171 (50)	171 (50)	$\chi^2 = 0.0, p = 1.00$
9-6 carriers, N (%)	26 (53)	23 (13)	$\chi^2 = 0.21, p = .65$
Male, N (%)	81 (57)	107 (48)	$\chi^2 = 0.0, p = 1.00$
Age in years, mean (SD)	17.14 (2.23)	16.56 (3.04)	$t(1, 485) = 1.75, p = .08$
	97.03 (14.24)	106.30 (13.53)	$t(1, 485) = -6.33, p < .001$
IQ, mean (SD)			
Inattentive scale, mean (SD) ^a	66.89 (11.69)	46.31 (5.75)	$t(1, 485) = 21.21, p < .001$
Hyperactive/impulsive scale, mean (SD) ^a	70.97 (14.80)	46.33 (5.05)	$t(1, 485) = 21.17, p < .001$
Total brain volume in ml, mean (SD) ^b	1240.73 (125.19)	1262.99 (123.13)	$t(1, 485) = -1.73, p = .09$

^a Measured with the Conners' Parent Rating Scale–Revised (Conners et al. 1998). Values refer to *t* scores on the DSM Total, Inattentive Behavior, and Hyperactive-Impulsive Behavior scales (scales N, L, and M).

^b Total brain volume is defined as the sum of total gray and white matter.

Supplementary Table 7. Striatal volumes and regression analyses testing differences between *DAT1* 10/10, 10-6, 9-6 carriers and non-carriers in a subsample of the NeuroIMAGE cohort, matched for gender and age

	NeuroIMAGE (N = 362)		Regression of binary genotypes on individual striatal volumes ^b
	<i>DAT1</i> 10/10 carriers (N = 221)	<i>DAT1</i> 10/10 non-carriers (N = 141)	
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	20.05 (0.09)	19.92 (0.12)	0.13; (-0.17;0.43), .39
	<i>DAT1</i> 10-6 carriers (N = 342)	<i>DAT1</i> 10-6 non-carriers (N = 20)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	19.97 (0.08)	20.51 (0.21)	-0.53 (-1.16;.10), .098
	<i>DAT1</i> 9-6 carriers (N = 49)	<i>DAT1</i> 9-6 non-carriers (N = 313)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	19.81 (0.20)	20.03 (0.79)	-0.23 (-0.65;.19), .29

^a Means are based on estimated marginal means corrected for age, gender, and total brain volume.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were diagnostic status, age, gender, and total brain volume.

Supplementary Table 8. Participant characteristics for the *DAT1* 10/10, *DAT1* 9-6 carriers, and non-carriers from the IMpACT cohort, matched for gender and age

Characteristics	IMpACT (N = 194)		Test of significance
	<i>DAT1</i> 10/10 carriers (N = 97)	<i>DAT1</i> 10/10 non-carriers (N = 97)	
Male, N (%)	9 (26)	69 (42)	$\chi^2 = 1.12, p = .29$
Age in years, mean (SD)	37.72 (12.18)	35.64 (11.00)	$t(1, 192) = 1.25, p = .21$
IQ, mean (SD)	109.25 (14.67)	107.61 (15.17)	$t(1, 192) = 0.77, p = .45$
Inattentive scale, mean (SD) ^a	3.31 (3.35)	4.04 (3.42)	$t(1, 192) = -1.50, p = .73$
Hyperactive/impulsive scale, mean (SD) ^a	3.02 (2.76)	3.58 (3.17)	$t(1, 192) = -1.31, p = .19$
Total brain volume in ml, mean (SD) ^b	1237.59 (114.58)	1237.04 (111.92)	$t(1, 192) = 0.34, p = .97$

Characteristics	IMpACT (N = 200)		Test of significance
	<i>DAT1</i> 9-6 carriers (N = 34)	<i>DAT1</i> 9-6 non-carriers (N = 166)	
Male, N (%)	9 (26)	69 (42)	$\chi^2 = 1.12, p = .29$
Age in years, mean (SD)	38.00 (13.26)	35.59 (10.77)	$t(1, 198) = 1.14, p = .26$
IQ, mean (SD)	110.65 (15.00)	109.50 (14.94)	$t(1, 198) = 0.41, p = .68$
Inattentive scale, mean (SD) ^a	4.76 (3.55)	3.28 (3.27)	$t(1, 198) = 2.37, p = .02$
Hyperactive/impulsive scale, mean (SD) ^a	4.12 (3.22)	2.98 (2.90)	$t(1, 198) = 2.05, p = .04$
Total brain volume in ml, mean (SD) ^b	1217.18 (99.67)	1252.57 (110.48)	$t(1, 198) = -1.73, p = .09$

^a Measured with the ADHD-DSM-IV Self Rating scale (Kooij et al., 2005).

^b Total brain volume is defined as the sum of total gray and white matter.

Supplementary Table 9. Striatal volumes and regression analyses testing differences between *DAT1* 10/10, 9-6 carriers, and non-carriers in the IMpACT cohort, matched for gender and age

	IMpACT (N = 194)		
	<i>DAT1</i> 10/10 carriers (N = 111)	<i>DAT1</i> 10/10 non-carriers (N = 89)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	18.76 (0.14)	18.86 (0.14)	-0.09; (-0.47;0.29), .64

	IMpACT (N = 200)		
	<i>DAT1</i> 9-6 carriers (N = 34)	<i>DAT1</i> 9-6 non-carriers (N = 166)	Regression of binary genotypes on individual striatal volumes ^b
	Mean (SE) ^a	Mean (SE) ^a	β (95% CI), <i>p</i> -value
Total striatum	19.74 (0.23)	18.71 (0.10)	1.03 (0.53;1.54), .0001 ^c

^a Means are based on estimated marginal means corrected for age, gender, and total brain volume.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were diagnostic status, diagnostic status * *DAT1* genotype, age, gender, and total brain volume.

^c $\beta = 1.03$ denotes that 9-6 carriers had a 1.03 ml larger striatum volume than non 9-6 carriers.

Supplementary Table 10. Striatal volumes and regression analyses testing differences between *DAT1* 9-6 carriers and non-carriers in the IMpACT cohort

	IMpACT (N = 229)		Regression of binary genotypes on individual striatal volumes ^b
	<i>DAT1</i> 9-6 carriers (N = 38)	<i>DAT1</i> 9-6 non-carriers (N = 191)	
	Mean (SE) ^a	Mean (SE) ^a	
Left accumbens	0.61 (0.015)	0.61 (0.007)	0.01 (0.03;0.04), .84
Right accumbens	0.55 (0.013)	0.52 (0.006)	0.03 (0.01;0.06), .04
Left caudate	3.81 (0.059)	3.61 (0.026)	0.20 (0.07;0.33), .002
Right caudate	3.92 (0.059)	3.70 (0.026)	0.22 (0.09;0.35), .001
Left putamen	5.52 (0.80)	5.20 (0.035)	0.32 (0.14;0.49), .0004
Right putamen	5.42 (0.072)	5.10 (0.032)	0.33 (0.17;0.48), .00005

^a Means are based on estimated marginal means corrected for diagnosis, age, gender, and total brain volume; for the NeuroIMAGE and BIG cohorts, covariates also included scanner type/location; for the BIG cohort, diagnostic status was dropped from the model.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were age, gender, and total brain volume.

Supplementary Table 11. Striatal volumes and regression analyses testing differences between *DAT1* 9-6 carriers and non-carriers for ADHD patients and controls separately from the IMpACT cohort

	IMpACT ADHD (N = 118)		Regression of binary genotypes on individual striatal volumes ^b
	<i>DAT1</i> 9-6 carriers (N = 26)	<i>DAT1</i> 9-6 non-carriers (N = 92)	
	Mean (SE) ^a	Mean (SE) ^a	
Left accumbens	0.64 (0.015)	0.61 (0.007)	0.03 (-0.01;0.06), .13
Right accumbens	0.54 (0.015)	0.51 (0.008)	0.04 (0.01;0.07), .05
Left caudate	3.83 (0.073)	3.60 (0.038)	0.24 (0.08;0.40), .005
Right caudate	3.94 (0.073)	3.67 (0.038)	0.26 (0.10;0.43), .002
Left putamen	5.56 (0.100)	5.14 (0.052)	0.42 (0.20;0.64), .0003
Right putamen	5.41 (0.087)	5.03 (0.045)	0.39 (0.19;0.58), .0001

	IMpACT Controls (N = 111)		Regression of binary genotypes on individual striatal volumes ^b
	<i>DAT1</i> 9-6 carriers (N = 26)	<i>DAT1</i> 9-6 non-carriers (N = 85)	
	Mean (SE) ^a	Mean (SE) ^a	
Left accumbens	0.57 (0.015)	0.60 (0.007)	0.03 (-0.10;0.03), .26
Right accumbens	0.55 (0.023)	0.53 (0.008)	-0.03 (-0.03;0.08), .30
Left caudate	3.76 (0.101)	3.62 (0.035)	0.14 (-0.07;0.35), .19
Right caudate	3.87 (0.104)	3.72 (0.036)	0.15 (-0.07;0.37), .18
Left putamen	5.36 (0.135)	5.26 (0.047)	0.10 (-0.18;0.39), .49
Right putamen	5.36 (0.127)	5.17 (0.044)	0.19 (-0.08;0.46), .16

^a Means are based on estimated marginal means corrected for age, gender, and total brain volume.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were age, gender, and total brain volume.

Supplementary Table 12. Regression of binary genotypes on total striatal volume corrected for lifetime medication use^a

	NeuroIMAGE (N = 487)	IMpACT (N = 229)
	β (95% CI), <i>p</i> -value ^b	β (95% CI), <i>p</i> -value ^b
10/10	0.21 (-0.06;0.48), .13	-0.17 (-0.53;0.20), .37
10-6	-0.40 (-0.70;0.38), .15	-0.41 (-1.05;0.22), .20
9-6	-0.27 (-0.63;0.09), .14	1.10 (0.63;1.56), .00001 ^c

^a Results from the final regression model examining associations between binary genotype (risk carriers vs non-risk carriers) and brain volumes.

^b For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in ml) between the genotype groups adjusted for covariates in the model. Included covariates were diagnosis, age, gender, total brain volume and lifetime medication use; for the NeuroIMAGE sample, covariates also included scanner type.

^c $\beta = 1.10$ denotes that 9-6 carriers had a 1.10 ml larger striatum volume than non 9-6 carriers.

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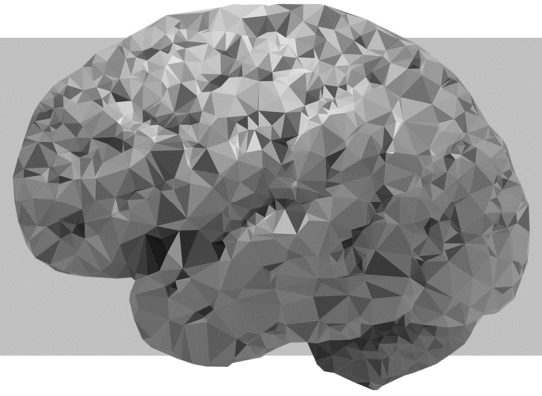
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7



Summary and General discussion

The work described in this thesis focused on neuroanatomical, neurocognitive and genetic factors involved in adult patients with Attention-deficit/hyperactivity disorder (ADHD). ADHD is characterized by cognitive and neurobiological abnormalities, such as executive functioning problems, reduced gray matter volume, and reduced white matter integrity of specific brain areas. ADHD is a neurodevelopmental disorder, which is highly heritable and in many cases affects an individual from childhood to adulthood. However, research on adults with ADHD continues to lag behind that on children with ADHD, in number and sample size (Ramos-Quiroga et al., 2014). For a better understanding of adult ADHD, it is imperative to know, whether a similar cognitive and neurobiological profile characterizes adults with ADHD. Therefore, the overall aim of this thesis was to gain more insight into the etiology of the persistent (adult) form of ADHD. The model of endophenotypes (Figure 1, *Chapter 1*) was used as a working model to study the relations between genes, endophenotypes of ADHD (either neuropsychological or neurobiological), and clinical features of the disorder. In this final chapter, the results from previous chapters will be summarized and integratively discussed within the context of current scientific literature. Their clinical implications will be discussed and how the results will influence future research.

Summary of the main findings

In *Chapter 2* of this thesis, the aim was to map the neuropsychological profile of adult patients with ADHD. There are numerous studies in childhood ADHD showing impaired functioning in multiple cognitive domains, most prominently in executive functioning (EF), reward processing, and timing. The few studies in adults with ADHD show similar impairments, but are hampered by small sample sizes and often only investigated a narrow range of neuropsychological measures. We therefore systematically examined neuropsychological performance on tasks measuring EF, delay discounting, time estimation, and response variability comparing adult patients with ADHD and healthy control participants. We also estimated the severity of neuropsychological dysfunctioning at the level of each individual. Our findings revealed that patients with ADHD showed impaired EF, were more impulsive, and were more variable in responding, compared to healthy adults. However, effect sizes were small to moderate, and 11% of patients did not show neuropsychological dysfunctioning. The best fitting model to predict ADHD from these neuropsychological data included measures from distinct cognitive domains (82.1% specificity, 64.9% sensitivity). We examined the effect of medication use and comorbidities on neuropsychological functioning, since most of the previous studies did not control for these potential confounders. We found that patients receiving stimulant treatment and those with a history of MDD were not distinctively impaired. To conclude, adults with ADHD resemble children with ADHD by being - as a group - impaired on several cognitive domains. Moreover, adult ADHD patients resemble children with ADHD in that they are a neuropsychologically heterogeneous group leading to large inter-individual differences.

In *Chapter 3* and *Chapter 4*, we moved from neuropsychological measurements to neurobiology, studying differences in gray and white matter volume (*Chapter 3*) and differences in white matter integrity (*Chapter 4*). The overall aim of these neuroimaging studies was to advance on previous findings in children with ADHD using the largest MRI/DTI sample in adult ADHD to date. Besides comparison between patients and controls, we investigated effects of gender, stimulant treatment, and a history of major depression (MDD). Studies in children show that ADHD is associated with smaller volumes of total brain matter and of subcortical regions, but it is unclear whether this represents delayed maturation or persists into adulthood. In *Chapter 3*, we compared brain volumes between adult ADHD patients and healthy control participants. We found no main effect of ADHD diagnosis on total volumes of gray and white matter and volumes of accumbens, caudate, globus pallidus, putamen, thalamus, amygdala and hippocampus. However, a significant gender by diagnosis interaction revealed that male patients showed reduced right caudate volume compared to male controls, and caudate volume in male patients was correlated with hyperactivity/impulsivity symptoms. Furthermore, medication-naïve patients showed larger right amygdala volume compared to patients using stimulant medication and controls. ADHD patients with episodes of MDD in the past showed smaller hippocampus volume compared to those with no history of MDD. The findings are consistent with the hypothesis of a brain maturation delay in ADHD. Reduced caudate volume in male patients may point to distinct neurobiological deficits underlying ADHD in the two genders. Smaller hippocampi in the patients with ADHD and a history of MDD is consistent with neurobiological alterations observed in MDD patients and suggests shared underlying biological etiologies.

Compared to imaging findings on brain volume, considerably less is known about white matter microstructure in adults with ADHD. In *Chapter 4*, we used Diffusion Tensor Imaging (DTI) to investigate white matter characteristics in adult ADHD patients and healthy control participants. Using a method called tract-based spatial statistics (TBSS) we investigated whole-skeleton changes of fractional anisotropy (FA) and mean, axial, and radial diffusivity (MD, AD, RD). Additionally, we studied the relation of FA and MD values with symptom severity and cognitive performance on tasks measuring working memory, attention, inhibition, and delay discounting. While we did not find differences in total white matter volume in the patients in *Chapter 3*, the integrity of white matter did appear to be altered in widespread areas of the brain. The results showed that patients with ADHD had reduced FA in corpus callosum, bilateral corona radiata, and thalamic radiation, when compared to controls. Higher MD and RD were found in overlapping and even more widespread areas in both hemispheres, also encompassing internal and external capsule, sagittal stratum, fornix, and superior lateral fasciculus. Values of FA and MD were not associated with symptom severity. However, within some white matter clusters that distinguished patients from controls, worse inhibition performance was associated with reduced FA and more impulsive decision making was associated with increased MD. Changes in RD suggest aberrant myelination as a pathophysiological factor in persistent ADHD. The

microstructural differences in adult ADHD may contribute to poor inhibition and greater impulsivity, but appear to be independent of disease severity.

In *Chapter 5* we revisited a finding from *Chapter 2*, which showed that patients with ADHD were more variable in responding as shown by increased standard deviation of reaction times (SDRT) on several neuropsychological tasks, compared to the healthy control participants. Instead of using standard statistics, such as SDRT, we chose in *Chapter 5* for an ex-Gaussian approach to reaction time analysis. An ex-Gaussian decomposition can handle skewed reaction time distributions and models them more accurately than a standard, Gaussian reaction time variability analysis. This analysis was also chosen based on a meta-analysis of reaction time variability in ADHD showing that increased SDRT in ADHD is largely driven by reaction times in the exponential tail of the distribution which is reflected by the ex-Gaussian parameter *tau* (Kofler et al., 2013). We focused on the hypothesis that attention lapses lie at the basis of increased *tau* (Leth-Steensen et al., 2000). Our aim was to investigate whether the microstructural integrity (measured as FA) of white matter was associated with *tau*. We defined regions of interest a priori and measured the mean FA of three main connecting fiber tracts from a right-hemispheric fronto-parietal attention network model implicated in ADHD. We found that increased *tau* was associated with an ADHD diagnosis and that *tau* was also linked to symptoms of inattention, supporting the attention lapse theory. An inverse correlation of *tau* with mean FA was seen in the right superior longitudinal fasciculus (SLF), but no direct association between the mean FA of the other tracts with ADHD could be observed. These findings suggest that the microstructural integrity of the right SLF contributes to elevated *tau* in ADHD.

In *Chapter 6*, a genetic imaging study was presented that combined genetic information and volumetric brain imaging. The dopamine transporter gene, *DAT1 (SLC6A3)*, has been studied extensively as a candidate gene for ADHD. Different variants of this gene (in particular those in the 3'untranslated region and in intron 8) are associated with childhood ADHD (10/10 & haplotype 10-6) and adult ADHD (haplotype 9-6). This suggests a differential association depending on age, and a role of *DAT1* in modulating the ADHD phenotype over the life time. The *DAT1* gene may mediate susceptibility to ADHD through effects on striatal volumes, where it is most highly expressed. To better understand how the *DAT1* gene exerts its effects on ADHD, the effect of three *DAT1* alleles (10/10 genotype, and the haplotypes 10-6 and 9-6) on striatal volume was studied in three cohorts, a childhood/adolescent sample (NeuroIMAGE, 301 ADHD patients and 186 healthy controls) and two adult samples (IMpACT, 118 patients with ADHD and 111 healthy controls; BIG, 1718 healthy participants). The results showed that only for the adults in the IMpACT cohort, carriers of the *DAT1* adult ADHD risk haplotype 9-6 had increased striatal volume relative to participants not carrying this haplotype. This effect varied by diagnostic status, with the risk haplotype affecting striatal volumes only in patients with ADHD. Consistent with this, the effect was not found in the BIG sample of adult healthy participants. It was also not observed in the case-control

children/adolescents sample from NeuroIMAGE. An explorative analysis in the cohorts combined (N=2434) showed a gene-by-diagnosis-by-age interaction suggests that carriership of the 9-6 haplotype predisposes to a slower age-related decay of striatal volume, which is specific for ADHD patients.

General discussion of findings

Inter- and intra-individual variability

One goal of this dissertation was to examine the neuropsychological deficits in adults with ADHD. We concluded that the deficits in adult ADHD resemble those observed in childhood. Despite statistically significant case-control differences, our results indicate a substantial degree of distributional overlap between ADHD patients and control participants. This means that no cognitive impairment has been identified as a specific marker for ADHD; the absence of cognitive dysfunctions does not rule out a diagnosis, whilst its presence does not guarantee one. Adult ADHD patients show large inter-individual differences in performance (which was also suggested by the moderate effect sizes from our case-control analyses) as was previously shown in children with ADHD (Coghill et al., 2014; Nigg et al., 2005). This clearly emphasizes that, besides reporting on a group level, researchers should report findings on an individual level by estimating for each individual the severity of neuropsychological dysfunctioning.

Our findings indicating *inter*-individual variability are in line with theories that there are distinct neuropsychological pathways leading to ADHD (Castellanos et al., 2006; Sonuga-Barke et al., 2010). Recently, these theories have found support from studies discerning subgroups of children with ADHD with distinct neurocognitive profiles (Coghill et al., 2014; Fair et al., 2012; Mostert et al., 2015; van Hulst et al., 2014).

Besides *inter*-individual variability, we also investigated *intra*-individual variability and found that ADHD patients were strikingly more variable in their performance than the healthy controls (*Chapter 2* and *Chapter 5*). For example, ADHD patients deviated most from controls on the measure that showed an increased variability in the number of errors over the course of a sustained attention tasks. Moreover, ADHD patients had, compared to controls, higher standard deviations of reaction time (SDRT) as measured on three reaction time tasks (out of four). Our strong results add to the growing empirical evidence signifying that increased reaction time variability (RTV) is a core feature of ADHD and is persistent over development. However, our current understanding of what specifically underlies increased RTV observations is still limited. Therefore, in *Chapter 5*, we dived deeper into the observations of increased SDRT by using an ex-Gaussian analysis of reaction times. Our findings revealed that the increased SDRT in ADHD patients was driven by reaction times in the exponential tail of the reaction time distribution, which is reflected by the parameter *tau*. Increased *tau* was associated specifically with symptoms of inattention, which fits with the hypothesis that linked increased *tau* to attention lapses (Leth-Steensen et al., 2000). We show that the use of the ex-Gaussian analyses of reaction times provides more interpretable results than using SDRT alone. While we specifically focused on the hypothesis that *tau* is

driven by attentional processes, there are many additional neural and physiological processes thought to be involved in increasing RTV, such as arousal, effort, and motivation (Karalunas et al., 2014b; Kofler et al., 2013). Future studies testing multiple competing theories within the same samples are therefore needed to better characterize mechanisms contributing to increased RTV.

Gray and white matter findings

The aim of *Chapter 3 and 4* was to investigate, whether adult ADHD patients have altered brain tissue volume and/or altered white matter characteristics, and how these abnormalities are associated with the clinical profile. We found no reductions in total gray and white matter volume in adult ADHD, when compared to controls (*Chapter 3*), which is consistent with previous studies (Amico et al., 2011; Biederman et al., 2008; Depue et al., 2010; Hesslinger et al., 2002; Monuteaux et al., 2008; Perlov et al., 2008; Pironti et al., 2014; Seidman et al., 2006). Besides the absence of differences in total volumes, we also did not find any reductions in subcortical volumes in the patients. The ADHD persistence model by Halperin and Schulz (2006), which predicts that the subcortical areas show relatively fixed or even progressive anomalies in ADHD across the lifespan, while cortical, prefrontal areas determine persistence or remission, was not supported by our data. Given that a reduction in total and regional brain volume is a consistent finding in children/adolescents with ADHD (Ellison-Wright et al., 2008; Frodl and Skokauskas, 2012; Greven et al., 2015; Nakao et al., 2011; Valera et al., 2007) our results support the maturational delay hypothesis that brain volume anomalies in ADHD largely disappear in the process of aging (Rubia, 2007). While our findings do contribute to a growing body of evidence implicating a delay in brain maturation in the pathophysiology of ADHD, they do not prove it. Although longitudinal studies suggest that a maturational lag is contributing to ADHD (Castellanos et al., 2002; Shaw et al., 2007; Shaw et al., 2012), there is still evidence from several studies of adults with childhood ADHD suggesting that gray matter abnormalities persist well into adulthood. These studies in adult ADHD patients show alterations in global gray and white matter volume (Maier et al., 2015) and in cortical thickness (McLaughlin et al., 2014; Shaw et al., 2006) and in some core areas like the basal ganglia as well as in cerebellar areas (Almeida Montes et al., 2010; Ellison-Wright et al., 2008; Frodl and Skokauskas, 2012; Makris et al., 2013; Proal et al., 2011). Importantly, since the effect sizes in adult ADHD imaging are small, most imaging studies in ADHD to date have been hampered in statistical power due to their limited sample sizes. To overcome sample size and statistical power, a recent study by the ENIGMA ADHD Working Group pooled all available data from the individual studies and examined subcortical and hippocampal brain volume differences between 1713 patients with ADHD and 1529 healthy individuals (Hoogman et al., submitted). The ENIGMA group found reduced striatal, amygdala, and hippocampal volumes in ADHD which were most pronounced in childhood supporting a model of delayed brain growth and maturation. They also confirm that effect sizes are small (ranging from $d = -0.08$ to $d = -0.15$) which clearly emphasizes the need for

well-powered imaging studies in order to find the subtle neuroanatomical differences between patients and control subjects.

The need for large samples is also underscored by our finding showing that ADHD male patients had smaller right caudate nucleus volumes than male controls, while there were no differences for females (*Chapter 3*). Earlier studies might have been somewhat underpowered to detect these subtle differences. However, more recent studies in comparably-sized or even larger samples (Greven et al., 2015; Hoogman et al., submitted; Maier et al., 2015) were unable to replicate our findings. This indicates that our finding described in *Chapter 3* might be a false positive finding, despite appropriate correction for multiple testing. Nonetheless, differences are present between males and females in brain volume (Rijkema et al., 2012) and in developmental trajectories for nearly all structures, with gray matter volume peaks generally occurring 1–3 years earlier in females (Lenroot et al., 2007). Further investigation of gender differences in ADHD may therefore be valuable since it can be an important factor accounting for (part of the) inconsistent findings in ADHD research. This is illustrated by a recent DTI study in children with ADHD showing sex-specific abnormalities in white matter (Jacobson et al., 2015). Boys appeared to be more affected in motor regions responsible for control of basic actions, whereas girls showed more abnormalities in prefrontal regions responsible for higher-level, top–down control.

As the study in *Chapter 3* showed that there were no significant differences in total white matter volume (or white matter macrostructure), we used DTI in *Chapter 4* to examine the microstructural characteristics of white matter in more detail. We found that primarily in the corpus callosum that fractional anisotropy (FA) of white matter was significantly decreased in the adults with ADHD relative to controls. Compared to the literature on volumetric imaging in ADHD, a limited number of studies have used the DTI technique, and there are currently no longitudinal DTI studies available. It is therefore difficult to draw conclusions on whether the maturational delay hypothesis which is defined for cortical gray matter can also be applied to the microstructural properties of white matter. However, our results are consistent with other DTI studies in adult ADHD suggesting that white matter abnormalities persist into adulthood (Cortese et al., 2013; Shaw et al., 2015). Our finding of reduced FA was driven by changes in radial diffusivity (RD) rather than axial diffusivity (AD), which is consistent with other recent findings in ADHD (Shaw et al., 2015; Witt and Stevens, 2015). The biophysical basis of RD is only partly understood, but includes reduced myelination. This can inform future pathophysiological studies and is a potential target for pharmacological and behavioral interventions. Future studies could benefit from a technique called Magnetization Transfer Imaging (MTI), which gives a more direct estimate of myelination than DTI (Wolff and Balaban, 1994). This technique has already been applied in a childhood ADHD study (de Zeeuw et al., 2012) and show that changes in frontostriatal connectivity in ADHD appear to be related to changes in microstructural organization rather than myelination per se.

Gene-brain-behavior relationships; the endophenotype model of ADHD

Neurocognitive heterogeneity has motivated the search for endophenotypes, which are those characteristics of a disorder that are linked relatively closely to its neurobiological substrates (Doyle et al., 2005)(see Figure 1, *Chapter 1*). In *Chapter 5* we used the concept of endophenotypes by examining specific brain – behavior relationships. We showed that reduced white matter integrity in the right SLF was associated with increased RTV and that this might drive presentations of inattention symptoms. Interestingly, we have shown in a previous magnetoencephalography (MEG) study using a spatial attention task that attention problems in adult ADHD patients arise from a failure in sustaining hemispheric alpha lateralization (ter Huurne et al., 2013). Recent findings from a MEG study in healthy participants showed that a larger SLF volume in the right compared to the left hemisphere (or vice versa) was associated with a better ability to modulate right compared to left hemisphere alpha and gamma band synchronization, with the latter also predicting biases in reaction time (Marshall et al., 2015). In future studies it would be very interesting to further test in ADHD patients whether the SLF is a structural pathway controlling alpha modulation as this could provide much new insight into the neurophysiological substrate of attention problems in ADHD.

While reduced FA in the right SLF is one of the most replicated DTI finding in ADHD (Cortese et al., 2013; Hamilton et al., 2008; Lawrence et al., 2013; Makris et al., 2008; van Ewijk et al., 2014), we found no differences in this structure, when following the traditional case-control analysis approach (*Chapter 4*). This shows that parsing the ADHD phenotype into a more meaningful phenotype, which is (potentially) closer to the neurobiology (e.g. RTV), leads to greater power to detect effects and better interpretable results concerning how a brain structure such as the right SLF is related to ADHD. In a further effort to reduce heterogeneity, we recently performed clustering analyses on the neuropsychological data. We found that both the ADHD and control group could be classified into three distinct profiles that differed in cognitive performance. Profile 1 was characterized by aberrant attention and inhibition, profile 2 by increased delay discounting, and profile 3 by atypical working memory and verbal fluency (Mostert et al., 2015). This study support previous clustering analyses performed in children (Coghill et al., 2014; Fair et al., 2012; van Hulst et al., 2014). A logical next step is to determine how the brain is affected in these separate neuropsychological subtypes of ADHD.

Additional examples of investigating brain–behavior relationships are found in *Chapter 3* and *4*. In *Chapter 3*, we showed that a decrease in caudate volume in male patients with ADHD was correlated with an increase in hyperactive-impulsive symptoms. Perhaps the model by Halperin & Schultz (2006), which proposes that subcortical dysfunction remains static throughout life, is only relevant for ADHD patients with the combined or hyperactive-impulsive subtype of ADHD. Such an effect could be mediated by gender, as males are more prone to have hyperactivity/impulsivity symptoms than females (Rucklidge, 2010). It would be very informative to see future longitudinal studies use dimensional approaches to further test this hypothesis. In *Chapter 4*, we used a dimensional

analysis to investigate, whether white matter disturbances were associated with symptom count. While two previous studies in children with ADHD showed that DTI parameters were driven by specific symptom domains (Shaw et al., 2015; van Ewijk et al., 2014), our dimensional approach resulted in non-significant findings. It can be concluded that the microstructural differences in adult ADHD appear to be independent of disease severity. Although we did find relations with neuropsychological measures suggesting that microstructural differences in adult ADHD may contribute to poor inhibition and greater impulsivity. In *Chapter 6*, striatal volume was used as an endophenotype, which opened the possibility to study how the *DAT1* gene exerts its effects on ADHD. The results of this study suggest different brain developmental trajectories in carriers of different *DAT1* alleles which might be specific for ADHD patients only and emphasize the need of a lifespan approach in genetic imaging studies of ADHD. Another fruitful area for volumetric research are multimodal approaches such as PET-MRI allowing researchers to benefit from both the molecular detail that PET provides plus the anatomic information provided by MRI. Knowing how *DAT1* expression levels are related to striatal volume might explain our hypothesis that larger striatal volume in adult carriers of the *DAT1* risk haplotype 9-6 for adult ADHD may represent compensatory mechanisms for the increased *DAT1* expression/activity in these individuals (and thus potential lack of dopamine signaling). While subcortical deficits are seen as a central feature of ADHD, the volumetric studies in *Chapter 4* and *6* highlight the complexity of how subcortical volume is related to ADHD. This thesis shows that consideration of gender, age, symptom dimensions, and genetic makeup are crucial for a full understanding. Therefore, longitudinal studies are needed to elaborate on our findings, which should take into account the interindividual differences (as indexed by the parameters above) on developmental trajectories of striatal brain areas in ADHD.

Rethinking the endophenotype model; the issue of specificity

The endophenotype model in this thesis helped us to provide a deeper understanding about what occurs on the pathway between genes and disorder. However, some caution is necessary here. One has to keep in mind that once we step away from a clinical diagnosis, the results could also become less specific and more general. For example, increased RTV, which was used in *Chapter 5*, is also found in other psychiatric disorders, such as Autism Spectrum Disorder (ASD), and thus lacks specificity for ADHD (Geurts et al., 2008). Such phenotypes that cut across existing diagnostic boundaries and/or relate to symptom dimensions expressed across multiple disorders are known as trans-diagnostic phenotypes (Nolen-Hoeksema and Watkins, 2011). Interestingly, a recent meta-analysis showed that increased RTV was only observed in ASD, when children with comorbid ADHD were included (Karalunas et al., 2014b). This suggests that increased RTV has a signal related to ADHD. Therefore, it may be informative to see dimensional studies that examine relationships between symptom dimensions and RTV in both disorders. Following our results, it could be that increased RTV is specific for patients that have high levels of ADHD attention symptoms, regardless of diagnostic assignment. Research concerning the possible etiologies of the co-

occurrence of ADHD and autism is scarce due to the previous DSM-IV diagnostic constraints (Rommelse et al., 2011). In the most recent version of the DSM-5, both disorders can co-occur which will hopefully spark new research including patients with both ASD and ADHD. Exciting recent neuroimaging studies reveal shared and disorder-specific structural and functional brain abnormalities (Dougherty et al., 2015; Lim et al., 2014; O'Dwyer et al., 2014; Ray et al., 2014).

Besides RTV, hippocampus volume can be seen as another example of a trans-diagnostic phenotype (*Chapter 3*). We showed that ADHD patients with a history of one or more major depressive episodes (MDD) had smaller hippocampi than patients with no MDD in their lifetime. A smaller hippocampus volume is a robust finding in depressive patients (Arnone et al., 2012). Following the same rationale that increased RTV in autism patients might signal an ADHD comorbidity, a smaller hippocampal volume in the patients with ADHD might reflect a common neurobiology with mood disorders (Du et al., 2012). Given that recurring depressive episodes occur in up to 50% of adults with ADHD (McIntosh et al., 2009), this finding provides more insight in the shared neurobiological features associated with depression. Altogether, our findings described in these studies support once more the significance of going beyond the diagnostic category of ADHD and also including other psychiatric disorders. Rather than increasing noise, it may increase signal, when certain circumscribed neural systems are targeted. The importance of shared neural substrates across psychopathology is illustrated by a recent meta-analysis of structural neuroimaging studies that showed reduced gray matter loss in the dorsal anterior cingulate, right insula, and left insula across multiple psychiatric diagnoses (Goodkind et al., 2015). Accepting that certain ADHD endophenotypes may cross diagnostic boundaries also has implications for the type of model that is assumed. By applying endophenotypes in different disorders, we will learn more about the specificity of genes, and neurobiological and behavioral defects that are shared and non-shared between disorders. This is also underscored by the new strategic plans of the U.S. National Institute of Mental Health (NIMH). In these plans, a strong emphasis is put on an approach known as the Research Domain Criteria (RDoC) approach (<http://www.nimh.nih.gov/research-funding/rdoc/index.shtml>). The explicit rationale for RDoC is to identify specific neural circuitry underlying typical and atypical behaviors and symptoms, with the goal of directing the search for treatment targets in multiple domains.

Strengths and limitations

The great strength of the analyses in this thesis is that they have been performed in a well-phenotyped sample; this enabled us to study ADHD at the multiple levels of the endophenotype model. The large sample size made it possible to study factors such as gender, comorbidity, and medication. While research on adults with ADHD continues to lag behind the research in children, the current thesis contributes to filling a gap in the neuropsychology and neuroimaging literature. In order to provide a full picture of the neurobiological profile of ADHD, we applied a broad battery of neuropsychological tests and several structural brain imaging measures.

However, some difficulties were also experienced and some limitations should be noted. First, the neuropsychological measures used in *Chapter 2* may not have been representative of the full spectrum of neurocognitive functions relevant for ADHD. While official diagnostic criteria for ADHD do not include any recognition of problems with emotional regulation, it is emerging as an important contributor to the ADHD phenotype (Karalunas et al., 2014a; Merwood et al., 2014; Sjowall et al., 2013). Second, there are potential confounding factors in our sample that require further investigation. The first is medication use in ADHD cases. Most ADHD cases in our sample had used medication during extended periods of time. While all participants were asked not to use their medication from 24 hours prior to testing, we had limited power to analyze differences between people having used medications and medication-naïve individuals. Therefore, there might still have been a confounding effect of medication through acute medication withdrawal and/or medication tolerance on neuropsychological functioning and on brain structure. Secondly, the presence of comorbid disorders may have introduced important confounds. Regardless of our sample size for studying case-control differences, effects of comorbidity could only be investigated for a limited set, and only the effects of a history of depression in ADHD patients could be analysed. Third, the applied design was cross-sectional, and therefore especially our findings regarding the effect of age on striatal volume in *Chapter 6* should be interpreted with caution. They should be replicated using a longitudinal design, which is the most optimal approach for studying the effects of age. Another point is the fact that we could not differentiate between remittent and persistent forms of ADHD. Neuropsychological and neuroimaging studies show that this distinction provides new insights in the prognosis of ADHD (Cortese et al., 2013; Proal et al., 2011; Van Lieshout et al., 2013). Fourth, we did not address environmental influences on neuropsychological and neuroimaging measures. Myelination begins early in the 3rd trimester of pregnancy, with the most rapid period of myelination occurring in the first two years of life. Therefore, prenatal exposure to maternal cigarette smoking or alcohol may be a prenatal factor of interest, when investigating white matter characteristics in ADHD (van Ewijk et al., 2015).

Clinical implications

Although this thesis, in general, addresses a more neurobiological – instead of a clinical – theme, there are some implications of this work that may be of interest to clinicians. Clinicians may take from these findings that ADHD patients should not generally be expected to present a severe neuropsychological impairment profile. It would be naïve to explain the etiology of ADHD to a client in terms of a single neuropsychological deficit, and clinicians should be aware of the existence of a group of patients with ADHD showing no neuropsychological deficits. Nevertheless, in addition to the standard interviews, neuropsychological profiling is still an important aspect of ADHD assessment and should involve a comprehensive assessment consisting of multiple tests measuring various aspects of cognition. Good neuropsychological assessment that reveals the strengths and weaknesses of a patient can provide targeted treatment such as working memory training

(Sonuga-Barke et al., 2013). Although the effects of training working memory are still being hotly debated (Melby-Lervag and Hulme, 2015).

Despite that several case-control differences have been reported for neuroimaging measures in this thesis, they cannot be used to objectively determine, whether someone suffers from ADHD. Recently, many investigators have expressed the hope that biological markers, such as neuroimaging measures, can be used to standardize and improve diagnostic assessment or prevent misdiagnosis (Adisetiyo et al., 2014; Faraone et al., 2014). Others state that it is unlikely that biomarkers will be soon used in clinical practice (Rommelse and de Zeeuw, 2014; Wolfers et al., 2015). The results from this thesis emphasize the large neurobiological heterogeneity within ADHD. Moreover, the lack of sensitivity and specificity at the neuropsychological and neurobiological level makes the identification of reliable and clinically useful biomarkers for diagnostic purposes unlikely. Nonetheless, endophenotypes have the potential to be used in a translational manner, thus allowing basic findings to influence practical applications. For example, the finding that white matter myelination is affected in patients with ADHD could inform applications such as repetitive Transcranial Magnetic Stimulation (rTMS). It has been shown in post-stroke aphasia patients that white matter integrity near rTMS stimulation sites showed increased FA which were driven primarily by reductions in RD (Allendorfer et al., 2012). While rTMS has been found effective in several psychiatric disorders (Reti, 2015), only one pilot study thus far used this application in ADHD and found positive effects on behavioral attention scores (Bloch et al., 2010). A stimulation site that could be of interest for ADHD is the attention network model as defined by Makris and coworkers (2009), which includes the right SLF. Studies investigating whether such rTMS stimulation can reduce RTV or enhance the ability to modulate right compared to left hemisphere alpha and gamma band synchronization (see above) will be an exciting area of new research on attention in ADHD. More research is clearly needed on how rTMS changes neuronal function, and whether it can be considered as a therapeutic tool and possible drug-free option for ADHD.

Key findings from this thesis

- Adult patients with ADHD, as a group, present similar neuropsychological performance characteristics as reported for childhood ADHD (*Chapter 2*).
- Adult ADHD is neuropsychologically heterogeneous, with different patients affected to a different degree by a range of distinct cognitive deficits and some patients not showing neuropsychological deficits at all (*Chapter 2*).
- Measures on which patients with adult ADHD deviated most from healthy controls were those reflecting performance variability or intra-individual variability. Results fit with the literature, which suggests that increased reaction time standard deviation in ADHD is driven by the ex-Gaussian parameter *tau* (*Chapter 2 and 5*).
- Adult patients with ADHD did not show a reduction in total brain volume compared to controls (*Chapter 3*).

- Male adult patients with ADHD had smaller right caudate nucleus volumes than male controls while there were no differences for females. Despite that it could be argued that this might be a false positive finding, investigation of gender differences is valuable, as gender can be an important factor accounting for (part of the) inconsistent findings in ADHD research (*Chapter 3*).
- Adult patients with ADHD had reduced fractional anisotropy in corpus callosum, bilateral corona radiata, and thalamic radiation, when compared to controls (*Chapter 4*).
- Reduced fractional anisotropy was driven by changes in radial diffusivity; this suggests that aberrant myelination is a pathophysiological factor in adult ADHD and might be a potential target for pharmacological and behavioral interventions or experimental interventions such as repetitive transcranial magnetic stimulation (*Chapter 4*).
- Reduced fractional anisotropy within the right Superior Longitudinal Fasciculus might underlie increased reaction time variability in ADHD, as was measured by elevated *tau* (*Chapter 5*).
- Carriership of the 9-6 haplotype predisposes to a slower age-related decay of striatal volume, which is specific for ADHD patients. This demonstrates the complexity of gene-by-diagnosis-by-age interactions in the brain and emphasizes the need for a lifespan approach in genetic studies of ADHD (*Chapter 6*).

Recommendations for future research: longer, larger, wider, and smarter

Based on the findings described and discussed in this thesis, suggestions for future research can be made on the basis of four keywords: longer, larger, wider, and smarter.

- Longer. Longitudinal studies are warranted as differences across the lifespan have been found in neuroimaging (Hoogman et al., submitted) and genetic results (Thissen et al., 2015), and provide a chance to study the potential role of such effects on persistence and remittance of ADHD. To address these issues, a follow-up study on the NeuroIMAGE cohort is currently underway.
- Larger. The average statistical power of studies in the neurosciences is generally very low (Button et al., 2013), and this hampers the replicability of findings from studies with small sample sizes. Because most effects of genes are also very small, large study samples are needed (Wu et al., 2014). To be able to obtain these large data sets, (international) collaborations have been created recently, such as ENIGMA (Thompson et al., 2014), the Human Connectome Project (Baroni and Castellanos, 2015), IMpACT (Franke et al., 2010), and the Psychiatric Genomics Consortium (Cross-Disorder Group of the Psychiatric Genomics, 2013).
- Wider. Future studies should widen their scope and examine endophenotypes across diagnostic boundaries to investigate, whether certain endophenotypes are specific for disorders or represent trans-diagnostic endophenotypes.
- Smarter. The field could profit from the use of more advanced analysis methods. For neuropsychology data, data-driven cluster approaches as performed in children

(Coghill et al., 2014; Fair et al., 2012; van Hulst et al., 2014) are largely still lacking in adult ADHD (see our recent study for a first example (Mostert et al., 2015). Besides improved neuroimaging techniques (e.g. allowing a finer level of spatial resolution), for (genetic) neuroimaging, multimodal and multivariate pattern analyses that take into account interactions between regions (i.e., brain structure or function patterns) are still lacking in adult ADHD (Liu and Calhoun, 2014; Mueller et al., 2013; Rubia et al., 2014) as well.

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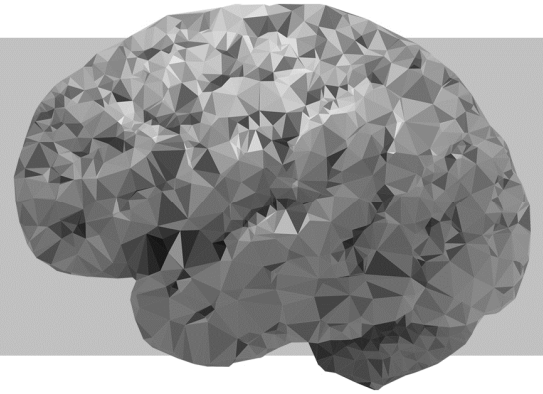
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Nederlandse Samenvatting

List of publications

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Curriculum Vitae

Donders Graduate School for Cognitive Neuroscience

Nederlandse Samenvatting

ADHD staat voor *Attention Deficit/Hyperactivity Disorder* en is een psychiatrische stoornis die zich in de kindertijd ontwikkelt. De symptomen die bij ADHD horen kunnen worden onderverdeeld in twee domeinen: symptomen van aandachtstekort en symptomen van hyperactiviteit/impulsiviteit. Om te voldoen aan de criteria voor een ADHD diagnose binnen de huidige classificatie van de DSM-5 moeten er 5 van de 9 symptomen van één van beide domeinen aanwezig zijn. Daarnaast gelden er nog een aantal andere criteria zoals het aanwezig zijn van de symptomen vóór het twaalfde levensjaar en aantoonbare beperkingen in meerdere situaties (school, thuis of op de sportvereniging). Hoewel ADHD bekend staat als stoornis in de kindertijd, vertoont ongeveer de helft van deze kinderen nog verscheidene ADHD symptomen in hun volwassen leven. In Nederland is aangetoond dat zelfs 70 procent van deze kinderen ook op volwassen leeftijd aan de diagnose blijven voldoen en wordt de prevalentie van ADHD bij volwassenen geschat op bijna drie procent. Het komt ook voor dat volwassen patiënten pas op latere leeftijd een ADHD diagnose krijgen. ADHD bij volwassenen uit zich op een iets andere manier dan in kinderen; de hyperactiviteit neemt af, maar de rusteloosheid neemt toe. De problemen die bij volwassen ADHD patiënten vaak voorkomen, zijn het vaak niet afmaken van een opleiding, of moeilijker een relatie en werk vinden en behouden.

Kinderen en volwassenen met ADHD hebben vaak comorbide stoornissen. Er is geschat dat rond de 75 procent van de volwassen patiënten naast ADHD nog een andere diagnose hebben zoals een depressie, verslaving of autisme. In 50 procent van de gevallen gaat ADHD samen met een depressie en is daarmee één van de diagnoses die het vaakst samengaat met ADHD. Bepaalde ADHD kenmerken, zoals concentratie en slaap problemen, komen ook voor bij depressie en dat maakt het soms moeilijk om een onderscheid te maken tussen de twee. Naast comorbiditeit speelt ook geslacht een rol bij ADHD. ADHD in de kindertijd komt twee tot drie keer vaker voor bij jongens dan bij meisjes. Bij volwassenen is dat meer gelijk en zijn er evenveel mannen als vrouwen met de stoornis.

Samenvattend kan gesteld worden dat de groep volwassen ADHD patiënten zeer heterogeen is door de diversiteit aan symptomen en de aanwezigheid van verschillende comorbide stoornissen. Dit maakt ADHD tot een complexe aandoening waarvan de onderliggende oorzaken nog voor een groot gedeelte onbekend zijn. Doordat nog niet precies bekend is hoe ADHD ontstaat en welke mechanismen er precies gestoord zijn is de behandeling van ADHD in de regel met medicatie (methylfenidaat, zoals bijvoorbeeld Ritalin of Concerta) en/of (cognitieve) gedragstherapie, vooral gericht op symptoombestrijding en niet op genezing.

De neurobiologie van ADHD

Uit de grote hoeveelheid onderzoek naar ADHD is bekend dat de stoornis een neurobiologische achtergrond heeft. In dit proefschrift wordt de neurobiologie van ADHD bij volwassenen onderzocht op verschillende niveaus waaronder genetica, hersenen en gedrag.

Het eerder onderzoek dat gedaan is bij ADHD op deze drie niveaus zal nu verder worden ingeleid en besproken.

De rol van genen bij ADHD

Voor een groot deel is ADHD erfelijk bepaald. Uit onderzoek met tweelingen is gebleken dat genetische factoren voor ongeveer 70 tot 80 procent verantwoordelijk zijn voor het ontstaan van ADHD. Ook zijn er enkele omgevingsfactoren bekend die de kans op ADHD verhogen, zoals alcoholgebruik tijdens de zwangerschap en een laag geboortegewicht. Bij de meeste ADHD patiënten spelen zowel genetische factoren als omgevingsfactoren een rol in het ontstaan van hun aandoening en daarom wordt ADHD een multifactoriële aandoening genoemd. Er is een combinatie van meerdere genetische factoren nodig, al dan niet samen met ongunstige omgevingsfactoren, voordat ADHD daadwerkelijk ontstaat. Daarnaast hebben de verschillende genetische factoren die een rol spelen bij multifactoriële aandoeningen vaak maar ieder een heel klein effect. Dit maakt het lastig om de genetische factoren voor ADHD te vinden. Door de toepassing van genomische opsporingsmethoden (GWAS: *Genome Wide Association Study*) zijn er slechts enkele genen geïdentificeerd voor ADHD. Naast deze hypothese vrije technieken zijn er op basis van bestaande neurobiologische theorieën enkele specifieke genen ontdekt die betrokken zijn bij ADHD. Voorbeelden hiervan zijn de dopamine genen *DAT1* en *DRD4* die de dopaminefunctie in het brein kunnen beïnvloeden, maar ook invloed kunnen uitoefenen op de neuroanatomie.

Neuroanatomische hersenverschillen bij ADHD

Uit eerder hersenonderzoek is duidelijk dat er afwijkingen bestaan in de hersenontwikkeling bij kinderen met ADHD. Een voorbeeld hiervan zijn verschillen in de hersenanatomie en kunnen in beeld worden gebracht met de techniek *Magnetic Resonance Imaging*, ook wel structurele MRI genoemd. De twee belangrijkste weefsels in het brein die zichtbaar gemaakt kunnen worden met deze technieken zijn de grijze en witte stof. De grijze stof bestaat uit de cellichamen van zenuwcellen die, simpel gezegd, het rekenwerk doen. De witte stof bevat de axonen die de verbindingen vormen tussen hersengebieden. Deze axonen zijn omwikkeld door myeline wat een wittige isolerende substantie is en bestaat uit vetten en eiwitten, vandaar de naam witte stof. Witte stof speelt een belangrijke rol in de informatieoverdracht in de hersenen.

In de afgelopen jaren is er veel onderzoek gedaan bij kinderen met ADHD waarbij er consistente aanwijzingen zijn dat kinderen met ADHD op groepsniveau een kleiner hersenvolume hebben. Daarnaast is gevonden dat bepaalde hersenkernen diep in het brein (zoals het striatum) verkleind zijn. Een belangrijke vraag is of deze afwijkingen bij kinderen met ADHD blijvend zijn of dat bij deze kinderen de hersenen later ontwikkelen en uiteindelijk op hetzelfde volume uitkomen als kinderen zonder ADHD. Er zijn inmiddels studies die aantonen dat er sprake is van het laatste en dat bepaalde delen van het brein (zoals de prefrontale cortex en het striatum) 3 tot 5 jaar vertraagd zijn bij patiënten met ADHD en uiteindelijk dus op hetzelfde volume uitkomen als mensen zonder ADHD. Echter, naast

studies die wijzen op een vertraagde ontwikkeling zijn er ook studies bij volwassenen met ADHD die blijvende reducties laten zien in hersenvolume.

Naast MRI, die hersenvolume van grijze en witte stof meet, kan men de microstructuur van de witte stof in beeld brengen met *Diffusion Tensor Imaging* (DTI). Een aantal eerste DTI studies laten zien dat de anisotropie (een maat voor de witte stof integriteit) van de witte stof verlaagd is bij kinderen met ADHD. Een verlaagde witte stof integriteit is gerelateerd aan een verminderde snelheid van informatieoverdracht. De resultaten wijzen erop dat de afwijkingen in kindertijd blijvend zijn en ook op volwassen leeftijd zichtbaar blijven, maar meer onderzoek is nodig. Vergeleken met de structurele MRI literatuur, is de DTI literatuur met betrekking tot ADHD nog beperkt en de kwaliteit van de studies is over het algemeen nog onvoldoende om sterke conclusies te kunnen trekken.

Voor zowel MRI and DTI zijn de resultaten bij volwassenen met ADHD nog inconsistent. Ook is het nog onduidelijk hoe verschillen in de hersenanatomie precies ontstaan. Er zijn wel aanwijzingen dat genetische factoren hierbij een rol kunnen spelen. Daarnaast is nog niet bekend hoe verstoringen in de grijze en witte stof, zoals een kleiner volume of verminderde witte stof integriteit, kunnen leiden tot neuropsychologische en gedragsproblemen die betrokken zijn bij ADHD.

De neuropsychologie bij ADHD

ADHD wordt vaak in verband gebracht met diverse neuropsychologische problemen. Deze cognitieve problemen kunnen grofweg worden ingedeeld in drie groepen: 1) problemen met de executieve functies waaronder aandacht, geheugen en impulscontrole; 2) motivationele problemen zoals het anders reageren op beloningen en straf en 3) een gestoorde temporele informatieverwerking (inschatten van tijd). Er is op dit vlak al veel onderzoek gedaan bij voornamelijk kinderen die laten zien dat de groep ADHD patiënten heel erg heterogeen is qua neuropsychologisch functioneren.

Naast de cognitieve problemen zoals hierboven besproken, blijkt een verhoogde reactietijd variabiliteit één van de meest robuuste fenomenen te zijn bij patiënten met ADHD. Dit betekent dat patiënten met ADHD meer variabel zijn in de manier waarop ze reageren tijdens neuropsychologische taken. De klassieke analyse van de reactietijd variabiliteit is op basis van de standaard deviatie van de reactietijden. Een nieuwere methode is de ex-Gaussian analyse die de reactietijd verdeling beschrijft middels drie parameters: *mu* (het gemiddelde van de normale verdeling), *sigma* (de standaard deviatie van de normale verdeling) en *tau* (het gemiddelde van het exponentiële deel). Het blijkt dat de verhoorde standaard deviatie van de reactietijden, zoals vaak gevonden in ADHD, wordt gedreven door reactietijden in het exponentiële gedeelte in de reactietijd verdeling (*tau*). Dit betekent dat tijdens computertaken, patiënten met ADHD vaker langzamer reageren dan mensen zonder ADHD. Een hypothese is dat tijdens dergelijke langzame reacties er sprake is van een tijdelijke terugval in de aandacht.

Vergeleken met het onderzoek dat gedaan is bij kinderen met ADHD is er bij volwassenen met ADHD aanzienlijk minder neuropsychologisch onderzoek gedaan en vaak in

kleine groepen proefpersonen. Hoewel reactietijd variabiliteit een robuust fenomeen is bij ADHD, zijn de onderliggende biologische mechanismen nog onbekend.

Endofenotypen

Zoals uit bovenstaande blijkt, is de neurobiologie van ADHD op meerdere niveaus erg complex en zijn er nog veel onbeantwoorde vragen. Om de complexiteit te verminderen en om beter te begrijpen hoe de verschillende niveaus van genetica, hersenen en neuropsychologie op elkaar inspelen, is het belangrijk om de relaties tussen deze niveaus te onderzoeken. Deze strategie wordt ook de endofenotype benadering genoemd. Endofenotypes zijn meetbare kenmerken die tussen de genetica (genotype) en het gedrag (fenotype) in liggen, zoals neurobiologische of neurocognitieve afwijkingen. Een voorbeeld van deze benadering zijn studies waarbij variatie in risico-genen voor ADHD, zoals het *DAT1* gen, worden gekoppeld aan volume verschillen in het brein (*imaging genetics*).

Doel van het proefschrift

Dit proefschrift heeft als algemene doel de genetische, neuro-anatomische en neurocognitieve mechanismen die ten grondslag liggen aan ADHD bij volwassenen te onderzoeken. Een eerste meer specifiek doel is om de ADHD literatuur in kinderen uit te breiden met studies in volwassenen met ADHD omdat naar deze laatste groep nog minder onderzoek is gedaan. In een grote groep volwassen ADHD patiënten en controle deelnemers is daarom in dit proefschrift op groepsniveau gekeken wat a) de verschillen zijn op een uitgebreide batterij van neuropsychologische maten (*Hoofdstuk 2*), b) de verschillen zijn in grijze en witte stof volumes gemeten met MRI (*Hoofdstuk 3*) en c) de verschillen zijn in witte stof integriteit gemeten met DTI (*Hoofdstuk 4*). In deze hoofdstukken wordt eveneens een aantal belangrijke factoren onderzocht die een rol kunnen spelen in de complexiteit van ADHD, zoals invloed van de comorbide stoornis depressie en de invloed van medicatie en geslacht op de onderzochte maten. Een tweede doel was om relaties tussen de neurobiologische niveaus te onderzoeken. Hierdoor werd het onderliggende mechanisme te onderzocht van de verhoogde reactietijd variabiliteit omdat daar nog weinig over bekend is (*Hoofdstuk 2 en 5*). Daarnaast werd de rol van het dopamine *DAT1* gen op hersenvolume onderzocht om meer inzicht te krijgen in hoe een gen het risico op ADHD verhoogt (*Hoofdstuk 6*).

Om bovenstaande onderzoeksdoelen te verwezenlijken werd de Nederlandse tak van het International Multicentre persistent ADHD genetics Collaboration (IMpACT) opgericht. Volwassen deelnemers met ADHD en zonder ADHD werden uitgenodigd om psychiatrische interviews te ondergaan en om bloed af te geven voor DNA analyse. Daarnaast werden neuropsychologische taken afgenomen en ondergingen deelnemers structurele en functionele MRI (*Hoofdstukken 2, 3, 4, 5, en 6*). Daarnaast is er gebruik gemaakt van de genetische en structurele MRI data van adolescenten met ADHD en zonder ADHD die hebben meegedaan aan het NeuroIMAGE onderzoek (*Hoofdstuk 6*). Tot slot is er gebruik

gemaakt van een grote groep gezonde deelnemers die hadden deelgenomen aan de Brain Imaging Genetics (BIG) studie. Dit is een langlopende en grootschalige studie naar de rol van genen op de hersenen (*Hoofdstuk 6*).

De bevindingen per Hoofdstuk samengevat

Hoofdstuk 2 beschrijft de resultaten waarbij verschillen in het neuropsychologisch functioneren werd onderzocht in een groep volwassenen met ADHD en in groep controle deelnemers zonder ADHD. Er werd gebruik gemaakt van een uitgebreide testbatterij waarbij verschillende domeinen werden onderzocht zoals executief functioneren, beloningsgevoeligheid, schatten van de tijd en de variabiliteit in reactievermogen. Op groepsniveau bleek dat volwassenen met ADHD lager scoorden op executief functioneren (waaronder werkgeheugen en aandacht), meer impulsief waren en meer variabel waren in hun reactietijden. De effecten waren klein en in overeenstemming met de verwachtingen, maar dit was nog niet eerder aangetoond in een grote groep volwassenen ADHD. Naast de groepsvergelijking zijn ook per individu de sterkte van neuropsychologisch disfunctioneren bepaald. Hieruit bleek dat er bij 11% van de patiënten geen sprake was neuropsychologisch disfunctioneren. Daarnaast is onderzocht hoe goed de testbatterij als geheel onderscheid kan maken tussen deelnemers met ADHD en zonder ADHD. Het beste voorspellende model bevatte een specificiteit van 82.1% en een sensitiviteit van 64.9%. Hoe hoger de specificiteit is, hoe beter de test mensen zonder ADHD kan aanwijzen. Hoe hoger de sensitiviteit is, hoe beter de test mensen met ADHD opspoort. Gebruik van medicatie en het doorgemaakt hebben van een depressie in het verleden hadden beide geen effect op onze resultaten. Concluderend lijken volwassen patiënten met ADHD, als groep, even aangedaan als kinderen met ADHD omdat ze uitval laten zien op dezelfde neurocognitieve domeinen. Ook de grote heterogeniteit in neuropsychologisch functioneren komt overeen met wat er gevonden is bij kinderen met ADHD. Deze resultaten suggereren verschillende cognitieve subtypen en zijn een verdere aanleiding tot neuropsychologische subtypering van ADHD.

In de *Hoofdstukken 3 en 4* werden neuroanatomische verschillen in grijze en witte stof onderzocht met behulp van MRI en DTI. *Hoofdstuk 3* beschrijft een structurele MRI studie waar hersenvolumes vergeleken werden tussen een groep volwassenen met ADHD en een groep controle deelnemers zonder ADHD. Volumes die vergeleken werden waren totale grijze en witte stof volume en volumes van diepere structuren in het brein zoals de accumbens, caudatus, globus pallidus, putamen, thalamus, amygdala en hippocampus. Uit de groeps-analyses bleek dat de hersenvolumes van patiënten met ADHD en gezonde controles niet verschilden van elkaar. Er was wel een interactie tussen diagnose en geslacht wat betekende dat bij mannen met ADHD de caudatus kleiner is wanneer deze werd vergeleken met mannen zonder ADHD. Het volume van de caudatus in mannen bleek daarnaast gerelateerd te zijn aan het aantal ADHD-symptomen. Hoe kleiner het volume, hoe meer impulsieve en hyperactieve symptomen de personen hadden. Er zijn geen verschillen gevonden in hersenvolume tussen vrouwen met ADHD en vrouwen zonder ADHD. Daarnaast werd er gevonden dat patiënten die stimulantia gebruikten (zoals Ritalin) de rechter

hippocampus kleiner was vergeleken met patiënten die geen medicatie gebruikten en controles. Daarnaast bleek dat patiënten die ooit een depressie hadden meegemaakt in hun leven een kleinere hippocampus hadden dan patiënten die nooit een depressie hadden meegemaakt. Dit werd eerder ook al gevonden in studies met depressieve patiënten. Alhoewel de deelnemers in deze studie niet longitudinaal zijn gevolgd van kindertijd naar volwassen leeftijd, lijken de resultaten erop te wijzen dat de hersenen bij ADHD patiënten op een later moment uitontwikkeld zijn. Een kleiner caudatus volume in alleen de mannen met ADHD wijst mogelijk erop dat de neurobiologische mechanismen die bij ADHD een rol spelen kunnen verschillen tussen mannen en vrouwen.

In *Hoofdstuk 4* werd de witte stof in het brein onderzocht met behulp van DTI. Uit de resultaten bleek dat de witte stof integriteit in het corpus callosum, de bilateral corona radiata en de thalamic radiation verminderd was bij de mensen met ADHD ten opzichte van de deelnemers zonder ADHD. Hierbij vonden we de grootste effecten in het corpus callosum wat ook de hersenbalk wordt genoemd. Deze zit in het midden van het brein en verbindt beide hemisferen met elkaar. De verminderde witte stof integriteit in de gebieden waar een verschil was gevonden was geassocieerd met verminderde neuropsychologische prestaties zoals slechtere respons inhibitie en verhoogde impulsiviteit. Daarnaast bleek dat specifiek myeline een rol leek te spelen bij deze verminderde witte stof integriteit.

In *Hoofdstuk 5* werd een bevinding uit *Hoofdstuk 2* opgevolgd welke liet zien dat de patiënten met ADHD meer variabel reageerden tijdens de computertaken (verhoogde standaard deviatie van de reactietijd). In *Hoofdstuk 5* is in plaats van de standaard deviatie van de reactietijd te gebruiken, een ex-Gaussian analyse uitgevoerd. Vergeleken met controle deelnemers, was *tau* verhoogd in de ADHD patiënten en geassocieerd met aandachtsymptomen. Een verhoogd *tau* (dus meer langere reactietijden) wordt geassocieerd met korte terugvallen in de aandacht. Dit betekent dat patiënten met ADHD het moeilijker lijken te vinden om de aandacht bij de computertaak te houden. Deze bevindingen werden vervolgens verder onderzocht en geassocieerd met de witte stof integriteit in het brein zoals gemeten met DTI. De hypothese was dat aandacht een rol speelt bij verhoogde *tau* en daarom is de witte stof integriteit in fronto-parietale netwerk gemeten waarvan gedacht wordt dat deze specifiek een rol speelt bij aandacht. Uit de analyse bleek dat een verminderde witte stof integriteit in de rechter superior longitudinal fasciculus gerelateerd was aan een hoger aantal langzame reacties tijdens de computertaak (*tau*). We vonden deze relatie bij zowel de deelnemers met als zonder ADHD. In de ADHD literatuur is verminderde witte stof integriteit in de rechter superior longitudinal fasciculus bij ADHD patiënten één van de meest gerepliceerde bevindingen. Het onderzoek in *Hoofdstuk 5* laat nu zien dat deze witte stof baan geassocieerd is met verhoogde *tau* en dus mogelijk een belangrijke rol speelt bij aandachtsprocessen.

Hoofdstuk 6 beschrijft het onderzoek naar één van de meest onderzochte genen in ADHD, het dopamine transporter gen (*DAT1*). Het 9-6 haplotype van het *DAT1* gen wordt vaker gevonden bij volwassenen met ADHD dan bij volwassenen zonder ADHD. Dit is opvallend omdat er bij kinderen met ADHD een ander risico haplotype van dit gen word

gevonden (10-6). Aangezien wordt verwacht dat het effect van het *DAT1* gen op ADHD klein is en verandert met leeftijd, werd er naast het cohort met volwassenen (IMpACT) ook gebruik gemaakt van een cohort met adolescenten met ADHD en zonder ADHD (NeuroIMAGE) en een cohort met alleen gezonde volwassenen zonder ADHD (BIG). Het *DAT1* gen komt voornamelijk tot expressie in het striatum. Het striatum speelt een grote rol in ADHD want het is betrokken bij beloning en straf welke beide gestoord zijn bij ADHD patiënten. Daarnaast worden ADHD patiënten vaak gekenmerkt door afwijkingen in activatie en grootte in het striatum. In deze studie is daarom het effect van het *DAT1* gen op het volume van het striatum onderzocht. Uit de resultaten bleek dat in de volwassen IMpACT cohort, dragers van het *DAT1* 9-6 haplotype een groter striataal volume hadden dan niet dragers. Dit effect van *DAT1* op striataal volume was alleen zichtbaar in volwassen patiënten met ADHD. Een exploratieve analyse waarbij alle cohorten samen werden genomen liet een 3-weg interactie zien tussen *DAT1* gen, diagnose en leeftijd. Dit suggereert dat de afname van striataal volume met leeftijd langzamer verloopt voor dragers van het 9-6 haplotype wat specifiek is voor ADHD patiënten.

Klinische implicaties

Ondank de hoofdzakelijke theoretische inslag, zijn uit dit proefschrift enkele hoofdbevindingen te herleiden die mogelijk indirect richting kunnen geven aan de klinische praktijk. Onderzoek in groepen zoals in dit proefschrift, waarbij patiënten worden vergeleken met controles, zegt helaas weinig over de toepasbaarheid van de resultaten voor één individuele patiënt. De resultaten geven wel duidelijk aan dat de groep met volwassen ADHD patiënten heterogeen is en dat klinici bewust moeten zijn van een groep patiënten die geen neuropsychologische uitval laat zien.

Net zoals bij de neuropsychologie, zijn de groeps effecten van ADHD op de hersenmaten klein. En net zoals bij de neuropsychologische maten, kunnen hersenscans (nog) niet gebruikt worden bij het stellen van een diagnose. Neuropsychologische testen zijn wel belangrijke klinische instrumenten bij het maken van sterkte-zwakte-profielen. De effecten van ADHD op de hersenmaten geven ons meer inzicht in de onderliggende mechanismen die bij ADHD een rol spelen. Bijvoorbeeld bij gevonden de verminderde witte stof integriteit bij ADHD, lijkt er een speciale rol van myeline te zijn. Dit wordt ook door andere onderzoeksgroepen gevonden en kan mogelijk een nieuw aangrijpingspunt zijn voor farmacologische en gedrags interventies, maar ook voor meer experimentele interventies zoals *repetitive Transcranial Magnetic Stimulation* ofwel herhaalde magnetische hersenstimulatie.

Kernbevindingen van deze thesis

- Volwassen ADHD patiënten hebben, als een groep, vergelijkbare neuropsychologische afwijkingen zoals gerapporteerd zijn in de kinder ADHD literatuur (*Hoofdstuk 2*).

- ADHD bij volwassenen is neuropsychologisch heterogeen wat betekent dat binnen de groep ADHD patiënten er grote verschillen zijn in de mate en aantal neuropsychologische afwijkingen waarbij er ook patiënten bestaan die helemaal geen afwijkingen laten zien (*Hoofdstuk 2*).
- De maten waar patiënten met ADHD het meest afweken van deelnemers zonder ADHD waren de maten die variabiliteit in de prestatie reflecteerden. Deze resultaten passen binnen de literatuur die suggereren dat verhoogde reactietijd variabiliteit in ADHD wordt gedreven door de ex-Gaussian parameter *tau* (*Hoofdstuk 2 en 5*).
- Tussen deelnemers met en zonder ADHD waren er geen verschillen in hersenvolumes (*Hoofdstuk 3*).
- Mannelijke volwassen ADHD patiënten hadden een kleinere rechter caudatus volume vergeleken met mannelijke controle deelnemers. Hoewel dit mogelijk een vals positieve bevinding kan zijn, is verder onderzoek naar geslacht effecten belangrijk omdat het een belangrijke factor kan zijn bij het verklaren van de inconsistente bevindingen in ADHD onderzoek (*Hoofdstuk 3*).
- Volwassen ADHD patiënten hadden verminderde witte stof integriteit in corpus callosum, bilateral corona radiata, en thalamic radiation vergeleken met deelnemers zonder ADHD (*Hoofdstuk 4*).
- Een gebrekkige myelinisering blijkt mogelijk ten grondslag te liggen aan de verminderde witte stof integriteit. Meer onderzoek is nodig om te kijken of myeline een belangrijk aangrijpingspunt is voor therapeutische interventies (*Hoofdstuk 4*).
- Verminderde witte stof integriteit in de rechter superior longitudinal fasciculus was geassocieerd met verhoogde reactietijd variabiliteit zoals gemeten met de ex-Gaussian parameter *tau* (*Hoofdstuk 5*) en speelt mogelijk een rol bij aandachtsprocessen.
- Volwassen dragers van het *DAT1* 9-6 haplotype hadden een groter striataal volume dan niet dragers. Afname van striataal volume met leeftijd lijkt langzamer te lopen voor dragers van het 9-6 haplotype wat specifiek is voor ADHD patiënten. Deze 3-wegs interactie geeft aan hoe gecompliceerd de genetische effecten op het brein zijn en benadrukt dat verder longitudinaal onderzoek nodig is (*Hoofdstuk 6*).

Toekomstig onderzoek

Samen met algemene bevindingen, kunnen op basis van de bevindingen in dit proefschrift suggesties voor toekomstig onderzoek gedaan worden aan de hand van vier kernwoorden: langer, groter, wijder en slimmer.

- Langer. Longitudinale studies zijn nodig omdat effecten van leeftijd zijn gevonden voor zowel structurele MRI/DTI resultaten als voor genetische bevindingen. Deze studies kunnen ook een kans bieden om beter te onderzoeken waarom kinderen met ADHD ook op volwassen leeftijd aan de diagnose blijven voldoen waarbij andere kinderen met ADHD op volwassen leeftijd niet meer voldoen aan de diagnose.

- Groter. De algemene statistische power van studies in de neurowetenschappen is klein wat ervoor zorgt dat resultaten moeilijk te reproduceren zijn. Daarnaast zijn genetische effecten erg klein waardoor grote groepen proefpersonen nodig zijn. Om aan deze grote aantallen te komen zijn recentelijk (internationale) samenwerkingen opgestart zoals *IMpACT*, *ENIGMA*, de *Human Connectome Project* en de *Psychiatric Genomics Consortium*.
- Breder. Toekomstige studies zullen hun blikveld moeten vergroten en endofenotypen onderzoeken in meerdere diagnoses tegelijk. Hierdoor kan bepaald worden of bepaalde endofenotypen diagnose specifiek zijn of bij meerdere diagnoses een rol spelen.
- Slimmer. Het onderzoeksveld kan profiteren van nieuwe geavanceerdere methodes. Voor de neuropsychologie kan men bijvoorbeeld gebruik maken van data-gedreven cluster analyses. Voor de MRI wordt de resolutie steeds beter, maar bestaan er ook multivariate methoden die interacties tussen gebieden meenemen en waar naar bepaalde patronen kan worden gezocht in structurele MRI scans.

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Diminished sensorimotor mu rhythm predicts hyperactivity and impulsivity severity in
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Curriculum Vitae

Marten Onnink was born in Winterswijk on the 13th of May 1983 and grew up in Aalten. In 2004 he started studying Psychology at the Radboud University Nijmegen. In 2008 he obtained his Master degree, *cum laude*, in Cognitive Psychology. In April 2009 he started with his PhD project at the department of Psychiatry of the Radboudumc, Nijmegen. In September 2013 he continued working as a researcher at the department of Human Genetics of the Radboud University Medical Center. In February 2015 he started teaching statistics and methodology courses for students from two bachelor's degree programs (Psychology and Pedagogy/Education) at the Vrije Universiteit of Amsterdam. In 2015 he obtained his University Teaching Qualification (UTQ). Besides teaching he works as a researcher for the department of Human Genetics of the Radboudumc, Nijmegen. Here, he is combining molecular genetic, neuroimaging, and neurocognitive research methods with the aim to identify mechanisms underlying associations between genes, brain, and cognition.

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